THE REMOVAL OF ROSE BENGAL FROM AQUEOUS SOLUTION BY RED BLOOD CELLS

by

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THE REMOVAL OF ROSE BENGAL FROM AQUEOUS SOLUTION BY RED BLOOD CELLS

During some work on photodynamic hemolysis[1,2] it was necessary to measure the amount of dye taken up by red blood cells from solution. It was found that the amount of dye taken depended only on the concentration of dye in solution, the number of cells being constant, and gave a straight line relationship. Jodbauer and Haffner[6] found the taking up of dye to follow the Freundlich Adsorption isotherm.

$$x = kc^{\frac{1}{n}}$$

$$\frac{1}{n} = 0.54$$

The data found in our preliminary experiments would not fit this adsorption equation. This research was undertaken to find if possible, the mechanism of the removal of dye from solution by red blood cells.

THEORETICAL

Three possible mechanisms for the removal of dye from solution by red blood cells are suggested. One would be adsorption. If it is assumed that the dye is adsorbed on the surface of the cells in a unimolecular layer, θ being the fraction of the surface covered by dye molecules, and $(1-\theta)$ the fraction not occupied, the rate of the dye molecules adsorbing on the surface at a concentration of c in the solution would be proportional to c and $(1-\theta)$. Then,

$$\text{Rate of molecules adsorbing on surface} = k_1 c(1-\theta).$$

The molecules leaving the surface would be proportional to the amount on the surface or the extent of surface coverage.

$$\text{Rate of molecules leaving surface} = k_2\theta.$$

At equilibrium the two rates will be equal. Hence,

$$k_1 c(1-\theta) = k_2\theta.$$

There are three conditions of surface coverage.

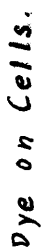
(a) If the surface is nearly bare. θ is small as compared to 1. $(1-\theta)$ becomes approximately 1. Then,

$$k_1 c = k_2\theta$$

rearranging

$$\theta = \frac{k_1}{k_2} c.$$

The fraction of the surface covered is proportional to the concentration in solution. The dye on the cells is directly proportional to the concentration of dye in solution. Represented graphically this would be



Dye in solution.

Figure 1.

This is true only if a small fraction of the surface is covered.

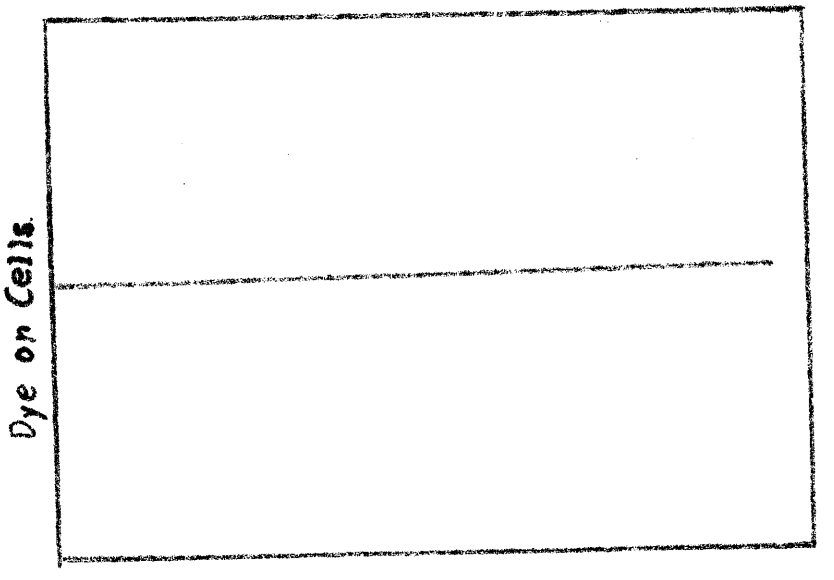
(b) The surface is nearly covered. θ is nearly 1. The variation in θ is small and the corresponding variation in $(1-\theta)$ is greatly magnified. An approximation would be

$$k_1 c(1-\theta) = k_2$$

$$1-\theta = \frac{k_2}{k_1 c}$$

Thus when the surface is nearly covered the extent of free surface is inversely proportional to the concentration of dye in solution. The surface covered and the amount of dye on the cells is nearly independent of the concentration of

dye in solution. Represented graphically this would be



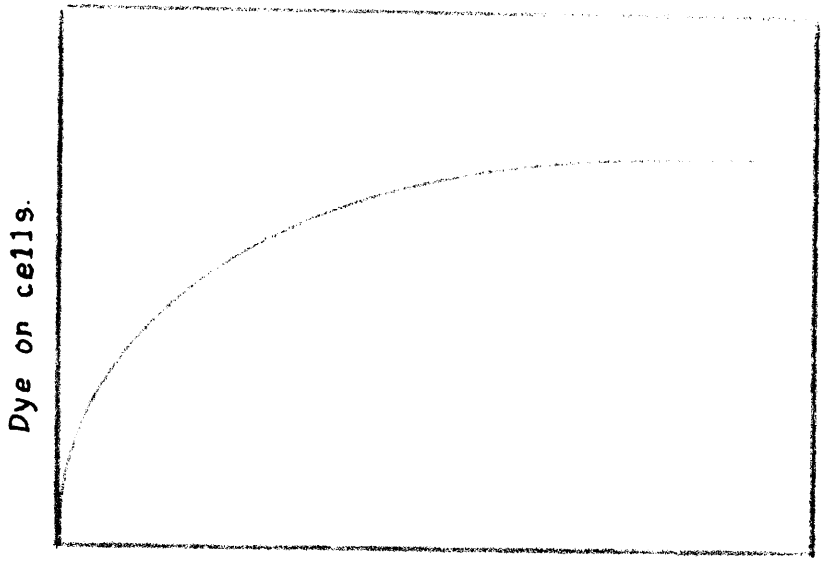
Dye in solution.

Figure 2.

(c) Where the surface is partially covered θ varies directly as c. θ varies as some power of c between 0 and 1. We may express this by the equation

$$\theta = kc^{\frac{1}{n}}$$

where $\frac{1}{n}$ is a fraction less than unity. This is the Freundlich adsorption isotherm. The relation may be expressed graphically



Dye in solution.

Figure 3.

A second mechanism is that the dye forms a chemical compound with some component of the cell membrane. b is assumed to be that component. If this reaction is irreversible there should be no dye left in solution as long as there is sufficient b to react with all of the dye.

$$mb + n\ \text{dye} \longrightarrow 1\ \text{b.dye (compound)}.$$

m and n are the number of molecules of b and dye which react to give 1 molecules of dye . b compound. If b is not sufficient to react with all of the dye there will be some value for the dye on the cells which corresponds to b being all reacted beyond which no more dye will be removed from solution by cells even though excess dye is present.

Where the reaction is reversible

$$m\ b + n\ dye \rightleftarrows 1\ b \cdot dye(compound).$$

In this case we have at equilibrium,

$$\frac{C^{l}_{b \cdot dye\ (compound)}}{C^{n}_{dye} \times C^{m}_{b}} = K$$

where C^{n}_{dye} , C^{m}_{b} , and $C^{l}_{b \cdot dye\ (compound)}$ are the concentrations of the molecules of dye, reacting substance b, and the compound. The concentrations of dye and dye compound (i.e. dye on the cells) are known. However, the concentration of the reacting substance is unknown and must be evaluated in some other manner. Also the order of the reaction, that is the number of molecules of each that react, is unknown.

If the concentration of b is assumed to be constant then

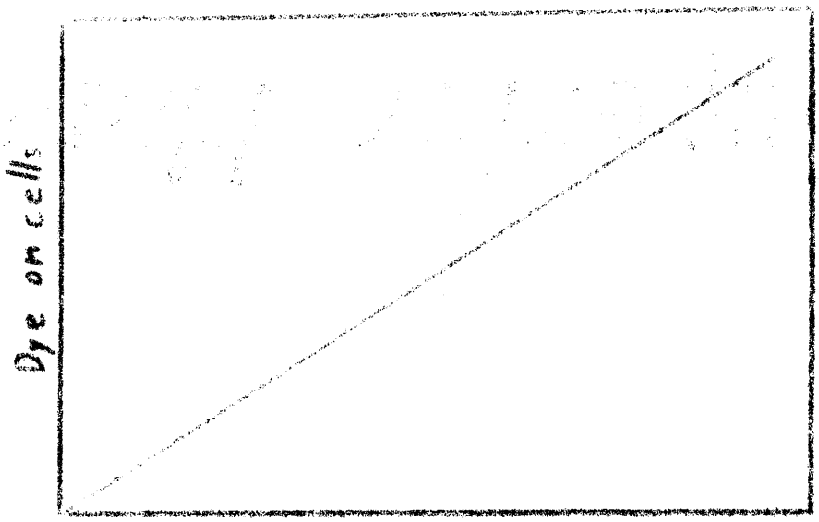
$$\frac{C^{l}_{b \cdot dye\ (compound)}}{C^{n}_{dye}} = C^{m}_{b}\ K$$

C^{m}_{b} is also constant. This becomes

$$\frac{C^{l}_{b \cdot dye(compound)}}{C^{n}_{dye}} = \text{Constant}$$

The dye on the cells is directly proportional to the dye in solution. This is true when the concentration of the reacting substance is very large compared to the amount reacted so that for all practical purposes it is a constant. That is, if only a small fraction of b is necessary for the reaction.

The third possible reaction is the distribution of the dye between immiscible solvents. The water solution acts as one solvent and the cell membrane as the second solvent. In this case the concentration of dye on the cells would be directly proportional to the concentration of the dye in solution if the molecular species is the same in both solvents, i.e. if there is no dissociation or association of the dye when it is transferred from one solvent to the other. This would be represented graphically as



Dye in solution.

Figure 4.

For a given concentration of cells the fraction of dye on the cells at equilibrium would always be the same.

If the reaction between the dye and the substance in the cell membrane is assumed to be one molecule of dye reacting with one molecule of substance forming one molecule of the compound of the two, some assumptions as to the kinetics of the reaction may be made. The rate in the forward direction is proportional to the concentration of dye and the concentration of the reacting substance.

$$\text{The forward rate } \frac{dc}{dt} = k_1(a-c)(b-c)$$

where a is the initial concentration of the dye, b is the initial concentration of the reacting substance in the cell membrane and c is the concentration of compound at time t. The rate of the reverse reaction is proportional to the concentration of the compound.

$$\frac{dc}{dt} \text{ reverse} = k_2 c$$

The rate of the reaction at any time short of equilibrium is the difference between the forward rate and the reverse rate.

$$\frac{dc}{dt} = \frac{dc}{dt} \text{forward} - \frac{dc}{dt} \text{reverse}$$

$$\frac{dc}{dt} = k_1(a-c)(b-c) - k_2 c.$$

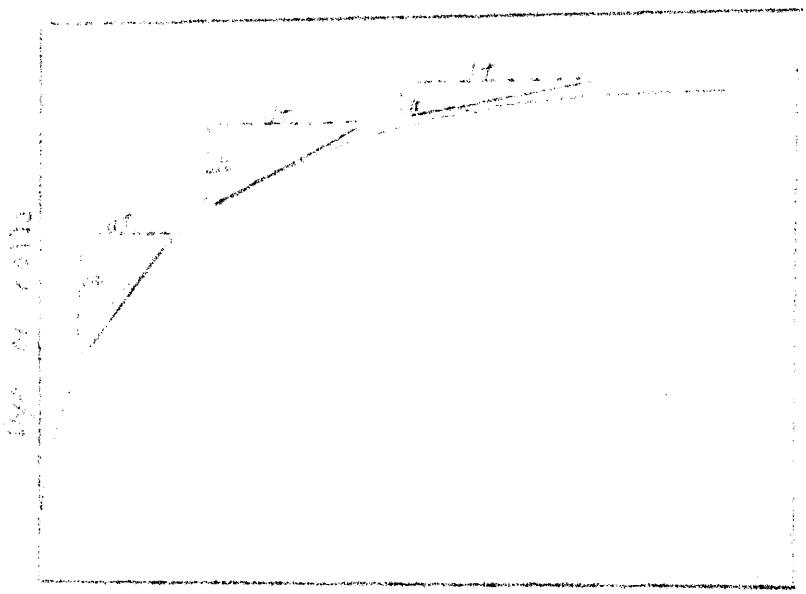
At equilibrium the two rates are equal.

$$k_1(a-c)(b-c) = k_2 c.$$

rearranging

$$\frac{c}{(a-c)(b-c)} = \frac{K_1'}{K_2}$$

By substituting the values of a, $\frac{dc}{dt}$, and corresponding values of c in these equations, b may be evaluated by simultaneous equations. $\frac{dc}{dt}$ at time t may be determined by the slope of the tangents to the time-concentration of dye on the cells curves.



Time in minutes.

Figure 5.

Corresponding values of c may be substituted in the equations

$$\frac{dc}{dt} = K_1(a-c)(b-c) - K_2c$$

and

$$\frac{c}{(a-c)(b-c)} = \frac{K_1'}{K_2}$$

By solving the simultaneous equations, K_1, K_2 and b may be determined.

The kinetics for adsorption and distribution would be nearly the same. The rate of molecules of dye passing from water solution to the membrane would be proportional to the amount in solution at time t.

$$\frac{dc_1}{dt} = K_1(a-c)$$

where a is the initial concentration of the dye in water solution and c is the concentration in the membrane. The reverse reaction, dye passing from the membrane to the water solution, would be proportional to the dye in the membrane.

$$\frac{dc_2}{dt} = K_2 c$$

At any time short of equilibrium the effective rate of movement of dye molecules to the membrane would be equal to the difference in these rates

$$\frac{dc}{dt} = \frac{dc_1}{dt} - \frac{dc_2}{dt}$$

$$\frac{dc}{dt} = K_1(a-c) - K_2 c$$

This same equation would apply to both adsorption and distribution.

The cell membrane is not a simple structure but has two components, the lipide or fatty portion and the protein. The dye may be soluble in both of these. This will give two positions for the dye in the membrane and the rates of the dye passing to and from these two portions may not be the same. If the dye passes directly from the water solution to

each with the membrane having a mosaic structure, we would have the following conditions.

$$\text{The forward rate } \frac{dc_1}{dt} = K_1(a-c_1-c_2)$$

and for the second component

$$\frac{dc_2}{dt} = K_2(a-c_1-c_2)$$

The forward rate is proportional to the concentration in solution at time t. Both concentrations in the cell must be subtracted from the initial concentration a. For the reverse reactions

$$\frac{dc_1}{dt} \text{ reverse} = K_3c_1$$

$$\frac{dc_2}{dt} \text{ reverse} = K_4c_2$$

The rate at any time will be the sum of the forward rates minus the sum of the reverse rates.

$$\frac{dc}{dt} = K_1(a-c_1-c_2) + K_2(a-c_1-c_2) - K_3c_1-K_4c_2$$

or $$\frac{dc}{dt} = (K_1+K_2)(a-c_1-c_2) - K_3c_1-K_4c_2$$

$c_1+c_2 = c$ = concentration of dye on cells.

$$= (K_1+K_2)(a-c) - K_3c_1 - K_4c_2$$

at equilibrium the rates are equal $\frac{dc}{dt} = 0$.

$$(K_1+K_2)(a-c) = K_3c_1+K_4c_2.$$

If the dye passes from the water solution to one component of the cell membrane and is in turn passed on to the second component there will be two consecutive reactions.

$$A \rightleftarrows C_1$$

$$C_1 \rightleftarrows C_2$$

The forward rates will be

$$\frac{dc_1}{dt} = K_1(a-c_1-c_2)$$

both c_1 and c_2 having been removed from the solution.

$$\frac{dc_2}{dt} = K_2(c_1-c_2)$$

where c_1 and c_2 are the concentrations of dye in the two parts of the membrane. The reverse rates are

$$\frac{dc_1'}{dt} = K_3c_1$$

$$\frac{dc_2'}{dt} = K_4c_2$$

The rate from solution to the first component would be

$$\frac{dc_1}{dt} = K_1(a-c_1-c_2) - K_3c_1$$

from one component to the other would be

$$\frac{dc_2}{dt} = K_1(c_1-c_2) - K_4c_2.$$

Again $c_1 + c_2 = c$ the concentration in the cells and at equilibrium the rates are equal.

It is not possible to calculate the values of the react-

-ions for the two cases without some knowledge of c_1 and c_2, the amounts of dye in the two parts of the membrane. Any reasonable value of c_1 and c_2 may be substituted and the constants may be adjusted to fit these values. The rates therefore do not give any indication of the values of c_1 and c_2.

EXPERIMENTAL

A darkened room was used for all experiments. Flasks containing cells and dye were only exposed in the room long enough to make transfers. They were kept in covered water baths to protect them from light. A very small amount of light for a short time did not seem to affect the cells but exposure to light for any length of time caused the cells to hemolyse. A red light did not cause hemolysis and, where it was possible, this light was used.

Each experiment was completed with one sample of blood. Blood from four rabbits was used. Cell counts were made with an hemocytometer to an accuracy of ±1 per cent. To give that accuracy about eight thousand cells were counted for each blood sample[7].

12 ml. of rabbit blood were collected in two tubes to which about .05 grams of sodium oxalate had been added as an anticoagulating agent. These tubes were used alternatly to collect the blood with vigorous shaking between each addition to thoroughly mix the blood and dissolve the sodium oxalate. To remove any small clots the blood was filtered through a small tuft of cotton and diluted to 60 ml. with sodium phosphate solution which was prepared according to Blum, Pace and Garret[3]. This solution was used in all dilutions of cells and dye.

The sodium phosphate buffer solution was prepared by diluting 90 ml. of 2 normal sodium hydroxide solution and 60 ml. of 2 molal phosphoric acid solution to one liter.

This gives a solution with a Ph. of 6.7 and the best osmotic pressure for red blood cells. The sodium hydroxide solution was standardized by titrating against weighed amounts of potassium acid phthalate. The phosphoric acid solution was standardized by titration with the sodium hydroxide solution using phenolphthalein and methyl orange as indicators. The methyl orange end point was checked with a solution of secondary potassium phosphate of the same concentration and containing the same amount of indicator. As a check a potentiometric titration was performed. This gave the same results as the indicator titrations.

The cells were spun down in a centrifuge. The supernatant solution was carefully drawn off with a pipette, care being taken not to disturb the red cells, and the cells were again made up to 50 ml. with fresh solution. This process was repeated until the cells had been washed four times to remove as nearly as possible all of the blood serum.

Washed cells were then made up to 275 ml. and 10 ml. portions were pipetted into each dye solution and blanks. Cells were added to buffer solution without dye and this solution was treated in the same manner as flasks containing cells. Blanks of cells and buffer were necessary as red blood cells even after numerous careful washings, still give off some material which absorbs light in the same part of the spectrum as rose bengal.

100 ml. volumetric flasks were used for the dilutions containing cells and the mixtures were kept in them until used or

discarded. All glassware was washed with warm sulfuric acid, rinsed twice with tap water, three times with distilled water, drained, and dried. Washing with sulfuric and chromic acid cleaning solution was avoided as this seemed to cause hemolysis of the cells.

A 10^{-3} molal solution was prepared from solid rose bengal. 25 ml. of this solution was diluted to 250 ml. for the 10^{-4} solution from which the 10^{-5} and 6×10^{-6} dilutions were made. 25 ml. of 10^{-4} solution was diluted to 250 ml. for the 10^{-5} dilution.

Cells will hemolyse if placed in solutions stronger than 1×10^{-5}. As the dye concentrations could not be measured with any degree of accuracy below 10^{-7} the range of starting dilutions were limited between 1×10^{-5} and 1×10^{-6}. 10 ml. of dye solution were pipetted into a 100 ml volumetric flask and diluted to within about 15 ml. of the mark. After shaking, the cells were pipetted into this solution and then diluted to the mark. This avoided the possibility of adding the cells to concentrated dye solution. All solutions were prepared some time before being used and allowed to stand in the water baths or refrigerator sufficient time to reach the desired temperature before adding the cells. A small amount of the buffer was also allowed to come to each temperature for dilution to the mark.

A thermostatically controlled electrically heated water bath was used for 35° c. This was controlled to about $\pm 0.1^{\circ}$c. A bath of flowing tap water was used for 25° c.

this was found to be within $\pm 0.3^{\circ}$ c. The greatest error in temperature was the fact that the solution rapidly warmed to room temperature, about 30° c., when placed in the centrifuge. However, most of the cells had been spun down at the start of centrifuging so this error was probably small.

After standing, with occasional shaking, the required time, samples were removed from the flasks and the cells centrifuged out. The clear dye solution was centrifuged a second time at a higher speed to remove all cells, debris, etc. A blank of cells with no dye was treated at the same time as each sample containing dye. [illegible] solution and blank were then measured in a spectrophotometer using a tube containing distilled water as a standard tube. Ten readings were taken on each solution. The tubes were reversed after five readings. The average of ten readings [illegible] average for the blank was subtracted to give the [illegible] photometer readings. A Bausch and Lomb visual spectrophotometer was used for the measurements. All were taken on the peak around 5461 A. This is the peak of the absorption band for rose bengal. The position of the line was determined by standardizing on the 5461 line from a mercury arc. The error in the spectrophotometer readings was about ± 3 per cent.

As Beer's Law, $k = \frac{1}{Lc}\log_e \frac{I_o}{I_t}$, was found to be accurate over the range of concentrations used, this was used to calculate the dye concentrations.

In order to check the reversibility of the reaction, 25 ml. of cells, prepared in the manner as in previous experi-

-ments, were suspended in 250 ml. of 10^{-5} dye at 25 ° c. and allowed to stand with an occasional very light shaking for three hours, then were spun down and resuspended in buffer The solution was treated as before to give velocity of the reverse reaction. A blank of cells and buffer solution were treated in the same manner. The equilibrium values for 1×10^{-6}, 5×10^{-6}, and 1×10^{-5} were determined on the same sample of blood. Results are given in Table IV.

In the experiments where the concentrations of the cells were changed, larger quantities of blood were necessary. It was found that the rabbits used could survive about a 20 ml. loss of blood. Two rabbits were used to get the required quantity of cells. The blood was kept separate until after the final washing to prevent possible clotting on mixing the blood. The blood was washed in the same manner as before and made up to a concentration of ten times that used in the previous experiments. 10 ml. of this solution was used for the 10 times blood concentrations, 5 ml. for the 5 times, and 10 ml. were diluted to 100 ml., and 10 ml. portions used for the same concentration as used in previous experiments.

The results are not as accurate where the larger quantities of blood cells are used. The greater concentration of cells increases the amount of hemolysis and also gives a much larger correction for the light absorbing substance form the cells. In the case of the concentration ten times the cell concentration used in previous experiments the amount of light absorbing substance gave a correction that exceeded by about ten times the reading attributed to the dye for 1×10^{-6} dye. In this

one instance the error in concentration of the dye may be ±50 per cent. Results are given in Tables V and VI.

The following outline is typical of these experiments. All solutions were prepared on the day before collecting the blood. 3-100 ml. flasks of dye and buffer were prepared for each temperature for the 1 x 10^{-6} dye concentrations. 10 ml. of 10^{-5} dye solution was added to each flask and then diluted with phosphate buffer solution to within about 15 ml. of the mark. Three flasks were used for the reaction at different times after mixing and one was used for equilibrium. Larger amounts of solution were necessary for this dilution as a 10 cm. absorption cell was used. 2 flasks of dye and buffer were prepared for the 5 x 10^{-6} dye concentration for each temperature. 5 ml. of 10^{-4} dye solution was added to each flask and then diluted. Similarly 2 flasks of dye and buffer were prepared for the 1 x 10^{-5} dye concentration for each temperature by diluting 10 ml. of 10^{-4} dye solution. 3-100 ml. flasks of buffer were prepared for the blanks for each temperature and 1 flask of buffer was prepared for the cell counts. These flasks were placed in their respective water baths and the refrigerator until used.

About 14 ml. of blood was collected from a small cut in the rabbits ear in two tubes. This blood was filtered through a small tuft of cotton and 12 ml. were diluted to 60 ml. in four 15 ml. centrifuge tubes. The cells were then spun down, the supernatant solution drawn off, and the cells diluted to 60 ml. with fresh solution. This was repeated until the cells

had been washed four times. They were then diluted to 275 ml. 10 ml. of this blood solution was added to each flask in its turn. The 1 x 10^{-6} dye concentration was run by itself as there were only six holderes in the centrifuge for tubes. 10 ml. of the blood solution were added to three 100 ml. volumetric flasks for one temperature. One was set aside in the bath and allowed to stand until the next day for the equilibrium value. After standing 10 minutes a 60 ml. sample was transferred to four 15 ml. centrifuge tubes and centrifuged 8 minutes. The supernatant solution was decanted and this solution centrifuged for 5 minutes. A flask containing only cells was treated at the same time as a blank. These solutions were decanted into a 10 cm. absorption tube and spectrophotometer readings were taken. This procedure was repeated at 30 minutes after mixing and 60 minutes after mixing. This was repeated for the other two temperatures.

The 5 x 10^{-6} and 1 x 10^{-5} dye concentrations were treated in the same manner. As a 1 cm. absorption cell was used for these dye concentrations, only a 30 ml. sample was necessary, and both solutions were run at the same time. These were treated the same as the 1 x 10^{-6} and the spectrophotometer readings obtained on each dye concentration and blank. Cells were added to the flasks for equilibrium at the same time. These were allowed to stand over until the next morning and were then centrifuged and the spectrophotometer readings taken.

CALCULATIONS

1. Dye concentrations from spectrophotometer readings:

Beer's Law $E = \frac{1}{LC} \log. \frac{I_o}{I}$

For two concentrations of the same substance:

$$\frac{1}{L_1 C_1} \log. \frac{I_o}{I} = \frac{1}{L_2 C_2} \log. \frac{I_o}{I}$$

The spectrophotometer readings are $\log. \frac{I_o}{I}$ Substituting readings from Table I.

$$\frac{1}{1 \times 10^{-5}} 0.781 = \frac{1}{10 \times C_2} 0.494$$

$$c_2 = 0.445 \times 10^{-6}$$

Results are given in Tables VII to XII inclusive.

2. Dye molecules per red blood cell.

Concentration of dye on cells 0.555×10^{-6} moles per liter from Table VII.

Red blood cells per liter:

2.275×10^4 per cubic millimeter or

2.275×10^{10} per liter.

Dye molecules per red blood cell:

$$\frac{0.555 \times 10^{-6} \times 6.06 \times 10^{23}}{2.275 \times 10^{10}} = 1.48 \times 10^7$$

Results are given in Tables XIII, XIV, and XV.

3. Molal Volume of Rose Bengal[5]

$C_{20} H_6 O_5 Cl_2 I_4 Na$

C_{20}	= 20 x 14.8	=	296.0
H_6	= 6 x 3.7	=	22.2
O' acid	= 12.0	=	12.0
O_4'	= 4 x 7.4	=	29.6
Cl_2	= 2 x 22.2	=	44.4
I_4	= 4 x 37.0	=	148.0
			552.2
Shrinkage for benzene rings:			44.1
			508.1

Volume of one molecule:

$$\frac{508.1}{6.06 \times 10^{23}} = 8.48 \times 10^{-22} \text{ cc.}$$

Assuming the molecules to be spheres:

$$\tfrac{4}{3}\pi r^3 = 8.48 \times 10^{-22}$$

$$\text{radius } r = 9.46 \times 10^{-8}$$

The sodium ion is not taken into consideration as it is probably not concerned in the reaction.

4. Surface covered by dye molecules.

The average surface of a rabbit red blood cell is about 80×10^{-8} sq. cm.[7]

Area of circle covered by dye molecule =

$\pi(9.45 \times 10^{-8})^2 = 8.95 \times 10^{-15}$

Fraction of surface covered by 1.93×10^{7} dye molecules

$$\frac{1.93 \times 10^{7} \times 8.95 \times 10^{-15}}{80 \times 10^{-8}} = 0.023$$

2.3 % covered.

5. Concentration of dye in the membrane.

Surface = 80×10^{-8} sq. cm.

The thickness of the membrane is about 160 AU.[8]

$80 \times 10^{-8} \times 160 \times 10^{-8} = 1.28 \times 10^{-12}$ c.c.

$1.28 \times 10^{-12}(2.275 \times 10^{10}) = .0281$ c.c.

in cell membranes of one liter of blood cells.

$\frac{7.71 \times 10^{-6}}{0.281 \times 10^{-8}} = 0.27$ molal solution extracted from

1×10^{-5} molal solution of dye in water.

6. If the reaction between the dye and the substance in the cell membrane is assumed to be a reversible bimolecular reaction

$$\frac{dc}{dt} = K_1(a-c)(b-c) - K_2c$$

where a is the initial concentration of dye in solution, b is the concentration of substance in the cell membrane which reacts with the dye. c is the concentration of dye on the cells at time t.

At equilibrium for the reaction

$$\text{Dye} + b \rightleftarrows \text{dye.b(compound)}$$

$$\frac{c}{(a-c)(b-c)} = \frac{K_1}{K_2} = K \text{ equilibrium constant}$$

c in this case would be the equilibrium concentration. By substituting values from Table XIII and $\frac{dc}{dt}$ from tangents to time-concentration curves(see Figures 5 and 10) the following simultaneous equations are obtained.

For 5×10^{-6} 0° C

(1) $.144\text{x}10^7 = K_1(15.9\text{x}10^7 - 6.52\text{x}10^7)(b-6.52\text{x}10^7) - 6.52\text{x}10^7K_2$

(2) $.0297\text{x}10^7 = K_1(15.9\text{x}10^7 - 7.58\text{x}10^7)(b-7.58\text{x}10^7) - 7.58\text{x}10^7K_2$

(3) $.008\text{x}10^7 = K_1(15.9\text{x}10^7 - 8.07\text{x}10^7)(b-8.07\text{x}10^7) - 8.07\text{x}10^7K_2$

at equilibrium.

(4) $$\frac{10.8 \times 10^7}{(15.9\text{x}10^7 - 10.8\text{x}10^7)(b - 10.8\text{x}10^7)} = \frac{K_1}{K_2}$$

By substituting equation (4) in (1) and (2) and solving the simultaneous equations for b.

$b = 13.8 \times 10^7$ for 5×10^{-6} initial dye concentration.

(4) may be substituted in (3) and the equation solved with (1) or (2) giving practically the same value for b.

$$b = 11.8 \times 10^7$$

In a similar manner the concentration of b may be determined for 1×10^{-6} dye concentration.

$$b = 2.87 \times 10^7$$

7. Calculations of reaction constants assuming that there is a monomolecular condensation of dye molecules into the cell membrane.

$$\frac{dc}{dt} = K_1(a-c) - K_2c$$

Using the average percentages from Table XVII, and the slopes drawn to curves of the concentration of dye on the cells with time. Figure 11.

equilibrium constant $K = \frac{K_1}{K_2}$

also $K = \frac{\text{dye on cells}}{\text{dye in solution}}$ at equilibrium

$K = \frac{76.2}{23.8} = 3.2$ $\frac{\text{on cells}}{\text{in solution}}$

$K_2 = \frac{K_1}{3.2}$

$1.09 = 49.5\ K_1 - \frac{50.15}{3.2} K_1$ for ten minute value.

K_1 = .0324 for ten minutes after mixing.

K_1 = .022 for thirty minutes after mixing.

K_1 = .009 for sixty minutes after mixing.

The average percentage values were used as they give the same results as the actual values and are more accurate as they reflect the average of a number of experiments.

TABLE I

Corrected Spectrophotometer Readings on the Supernatant Dye Solutions.

Temperature	Initial dye concentration	Corrected Spectrophotometer Readings on Solution 10 min.	30 min.	60 min.	6 hours
35° C	1×10^{-6}	.0347	.0261	.0217	H
	5×10^{-6}	.187	.120	.105	H
	1×10^{-5}	.346	.255	.242	H
25.5°	1×10^{-6}	.0341	.0307	.0271	H
	5×10^{-6}	.212	.168	.149	H
	1×10^{-5}	--	--	--	.179
0° C	1×10^{-6}	.0494	.0499	.0444	.0336
	5×10^{-6}	.253	.218	.208	.120
	1×10^{-5}	.543	.466	.396	.327

1×10^{-5} dye with no cells 0.781

Red Blood Cell Count = 2.275×10^{-4}

H indicates hemolysis of sufficient cells to make spectrophotometer readings inaccurate. It was not necessary for all of the cells to hemolyse to cause this difficulty. The hemoglobin released when the cell membranes broke caused enough scattering and absorption of the light to make readings impossible.

TABLE II

Corrected Spectrophotometer Readings on the Supernatant Dye Solutions

Temperature	Initial Dye Concentration	Corrected Spectrophotometer Readings on Solution 10 min.	30 min.	60 min.	6 hours
35°C	10^{-6}	.0270	.0216	.0218	H
	5×10^{-6}	.161	.114		H
	10^{-5}	.328	.311	.312	H
25.5°C	10^{-6}	.0353	.0243	.0176	H
	5×10^{-6}	.196	.149	.111	H
	10^{-5}	.394	---**	.309	H
0°C	10^{-6}	.0414	.0357	.0300	.0142
	5×10^{-6}	.230	.230	.192	.126
	10^{-5}	.507	.419	.404	.331

1×10^{-5} dye with no cells 0.78

Red Blood Cell Count = 1.907×10^{4}

**Absorption cell for spectrophotometer was broken and the solution was lost.

TABLE III

Corrected Spectrophotometer Readings on the Supernatant Dye Solutions

Temperature	Initial Concentration	Corrected Spectrophotometer Readings on Solution: 10 min.	30 min.	60 min.	6 hours
25°C	10^{-6}	.0357	.0278	.0244	.0175
	5×10^{-6}	.208	.148	.097(?)	.113
	10^{-5}	.419	.304	.246	.206

10^{-5} dye with no cells .78

Red Blood Cell Count = 1.97×10^{4}

TABLE IV

Corrected Spectrophotometer Readings for the Reverse Reaction

Time after remixing	Corrected Spectrophotometer reading
10 minutes	.0239
30 minutes	.0357
60 minutes	.0463
120 minutes	.0619
180 minutes	.0794
420 minutes	.1302
1×10^{-6} equilibrium	.0165
5×10^{-6} equilibrium	.128
1×10^{-5} equilibrium	.232

10^{-5} dye with no cells 0.78

Red Blood Cell Count = 1.675×10^{4}

Temperature 24.0° C

TABLE V

Corrected Spectrophotometer Readings for Different Concentrations of Red Blood Cells

Initial dye Concentration	Concentration of cells	Corrected Spectrophotometer Readings			
		10 min.	30 min.	60 min.	6hours
10^{-5}	10 x R.B.C.	.234	.186	.149	.113
	3 x R.B.C.	.284	.198	.158	.134
	1 x R.B.C.	.387	.301	.252	.231

10^{-5} dye with no cells .731

Red Blood Cell Count = 2.2×10^4

TABLE VI

Corrected Spectrophotometer Readings for Different Concentrations of Red Blood Cells.

Concentration of cells	Initial dye Concentration	Corrected Spectrophotometer Readings 60 min.	5 hours
10 x R.B.C.	10^{-6}	.008	.009
	5×10^{-6}	.044	.042
	10^{-5}	.057	.063
5 x R.B.C.	10^{-6}	.029	.028
	5×10^{-6}	.056	.054
	10^{-5}	.111	.100
1 x R.B.C.	10^{-6}	.027	.029(?)
	5×10^{-6}	.108	.097
	10^{-5}	.257	.223

1×10^{-5} dye with no cells .741

Red Blood Count = 1.826×10^{4}

TABLE VII

Concentration of Dye on the Cells and Dye in Solution

Concentrations are moles x 10^{-6} per liter of solution or cells

Temp.	Initial concentration	Time after mixing: 10 minutes		30 minutes		60 minutes		Equilibrium	
		Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution
35°C	1 x 10^{-6}	0.555	0.445	0.666	0.334	0.723	0.277	H	H
	5 x 10^{-6}	2.51	2.49	3.465	1.535	3.655	1.345	H	H
	1 x 10^{-5}	5.57	4.43	6.73	3.27	6.90	3.10	H	H
25.5°C	1 x 10^{-6}	0.563	0.437	0.607	0.393	0.653	0.347	H	H
	5 x 10^{-6}	2.28	2.72	2.85	2.15	3.69	1.31	H	H
	1 x 10^{-5}							7.71	2.29
0°C	1 x 10^{-6}	0.367	0.633	0.361	0.639	0.432	0.568	.557	.433
	5 x 10^{-6}	1.75	3.25	2.08	2.92	2.34	2.66	3.46	1.54
	1 x 10^{-5}	3.05	6.95	4.05	5.95	4.92	5.08	5.81	4.19

Red Blood Cell Count = 2.275 x 10^{4} per cu. mm.

TABLE VIII

Concentrations of Dye on the Cells and Dye in Solution

Concentrations are moles x 10^{-6} per liter of solution or cells.

Temp.	Initial concentration	Time after mixing: 10 minutes		30 minutes		60 minutes		Equilibrium	
		Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution
3 5°C	1 x 10^{-6}	0.654	0.346	0.723	0.277	0.722	0.278		
	5 x 10^{-6}	2.94	2.06	3.54	1.46	-	-		
	1 x 10^{-5}	5.80	4.20	6.02	3.98	6.00	4.00		
25.5°	1 x 10^{-6}	0.548	0.452	0.689	0.311	0.733	0.226		
	5 x 10^{-6}	2.49	2.51	3.09	1.91	3.58	1.42		
	1 x 10^{-5}	4.95	5.05	-	-	6.04	3.96		
0°C	1 x 10^{-6}	0.47	0.53	0.542	0.458	0.616	0.384	0.818	0.182
	5 x 10^{-6}	2.05	2.95	2.385	2.615	2.54	2.46	3.385	1.615
	1 x 10^{-5}	3.50	6.50	4.63	5.37	4.82	5.18	5.76	4.24

Red Blood Cell Count = 1.907 x 10^4

TABLE IX

Concentrations of Dye on the Cells and Dye in Solution

Concentrations are moles x 10^{-6} per liter of solution or cells.

Temp.	Initial concentration	Time after mixing							
		10 minutes		30 minutes		60 minutes		360 minut s	
		Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution
25°C	1 x 10^{-6}	0.551	0.449	0.644	0.356	0.688	0.312	0.776	0.224
	5 x 10^{-6}	2.34	2.66	3.15	1.85	3.76	1.24	3.555	1.445
	1 x 10^{-5}	4.64	5.36	6.11	3.89	6.85	3.15	7.36	2.64

Red Blood Cell Count = 1.97 x 10^{4}

TABLE X

Concentrations of Dye on the Cells and Dye in Solution for Reverse Reaction

Initial Dye Concentration = 1×10^{-5}

Concentrations are moles $\times 10^{-6}$ per liter of solution or cells.

24° C. R.B.C.Count = 1.675×10^4

Time after remixing	Dye on cells	Dye in solution
0 minutes	7.03	0
10 minutes	6.724	0.306
30 minutes	6.570	0.460
60 minutes	6.437	0.593
120 minutes	6.237	0.793
180 minutes	6.014	1.016
420 minutes	5.463	1.667
Equilibrium Values		
10^{-6}	.788	0.212
5×10^{-6}	3.36	1.64
10^{-5}	7.03	2.97

TABLE XI

Concentration of Dye on Cells and Dye in Solution with Changing Concentrations of Cells

Initial dye concentration 10^{-5}

Concentrations are moles x 10^{-6} per liter of solution or cells.

Concentration of cells	Time after mixing							
	10 minutes		30 minutes		60 minutes		360 minutes	
	Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution	Dye on cells	Dye in solution
10 X	6.80	3.20	7.46	2.54	7.96	2.04	8.45	1.55
3 X	6.13	3.87	7.29	2.71	7.83	2.17	8.16	1.84
1 X	4.70	5.30	5.87	4.13	6.55	3.45	6.83	3.17

Red Blood Cell Count = 2.2 x 10^4

TABLE XII

Concentrations of Dye on Cells and Dye in Solution with Changing Concentrations of Cells

Concentrations are moles x 10^{-6} per liter of solution or cells.

Initial dye concentration	Concentration of cells	Time after mixing: 60 minutes: Dye on cells	60 minutes: Dye in solution	360 minutes: Dye on cells	360 minutes: Dye in solution
10^{-6}	10 X	0.892	0.108	0.879	0.121
5×10^{-6}	10 X	4.406	0.594	4.434	0.566
10^{-5}	10 X	9.232	.768	9.150	0.850
10^{-6}	5 X	0.609	0.391	0.622	0.378
5×10^{-6}	5 X	4.245	0.755	4.272	0.728
10^{-5}	5 X	8.505	1.495	8.65	1.35
10^{-6}	1 X	0.646	0.361	0.609	0.391
5×10^{-6}	1 X	3.545	1.455	3.693	1.307
10^{-5}	1 X	6.53	3.47	6.97	3.03

Red Blood Cell Count = 1.825×10^{4}

TABLE XIII

Dye Molecules per Red Blood Cell

Dye concentrations are dye molecules x 10^7 per cell

Temp.	Initial concentration per red blood cell.	Dye on cells: Time after mixing: 10 min.	30 min.	60 min.	Equilibrium
35°C	2.66	1.48	1.78	1.93	
	13.3	6.68	9.24	9.74	
	26.6	14.85	17.66	18.4	
25.5°	2.66	1.50	1.62	1.74	
	13.3	6.08	7.60	9.84	
	26.6				20.53
0°C	2.66	0.98	0.96	1.15	1.51
	13.3	4.67	5.55	6.24	9.23
	26.6	8.13	10.8	13.1	15.5
35°C	3.18	2.08	2.30	2.30	
	15.9	9.34	11.26		
	31.8	18.46	19.2	19.1	
25°C	3.18	1.74	2.19	2.46	
	15.9	7.94	9.82	11.4	
	31.8	15.75		19.2	
0°C	3.18	1.50	1.72	1.96	2.6
	15.9	6.52	7.58	8.07	10.8
	31.8	11.14	14.72	15.32	18.3
25°C	3.07	1.69	1.98	2.11	360 minutes 2.39
	15.36	7.16	9.68	11.55	10.9
	30.72	14.26	18.8	21.1	22.9

TABLE XIV

Dye Molecules per Red Blood Cell - Reversal Reaction

Time after mixing	Dye on cells
0 minutes	25.45×10^7
10 minutes	24.32
30 minutes	23.78
60 minutes	23.28
120 minutes	23.58
180 minutes	21.78
420 minutes	19.8

Equilibrium Values

Initial dye per R.B.C.	Dye on R.B.C.
3.62×10^7	2.85×10^7
18.1	12.15
36.2	25.45

TABLE XV

Dye Molecules per Red Blood Cell

Changing Concentration of Cells.

Initial concentration	Time after mixing			
	10 minutes	30 minutes	60 minutes	360 min.
2.85×10^7	1.94×10^7	2.13×10^7	2.27×10^7	2.41×10^7
9.17	5.82	6.82	7.43	7.74
28.5	13.40	16.75	18.70	19.50
0.332			0.296	0.298
1.66			1.46	1.47
3.32			3.06	3.04
.664			.408	.413
3.32			2.32	2.84
6.64			5.64	5.74
3.32			2.14	2.02
16.6			11.78	12.27
33.2			21.68	23.12

TABLE XVI

Percentage of Surface Covered by Dye Molecules

Temp.	Initial dye concentration	Time after mixing: 10 minutes	30 minutes	60 minutes	Equilibrium
35°C	1 x 10^{-6}	1.8%	2.1 %	2.3 %	
	5 x 10^{-6}	7.3	11.1	11.7	
	1 x 10^{-5}	17.7	21.2	22.0	
25°C	1 x 10^{-6}	1.8	1.9	2.1	
	5 x 10^{-6}	7.2	9.1	11.8	
	1 x 10^{-5}				24.5 %
0°C	1 x 10^{-6}	1.2	1.2	1.4	1.8
	5 x 10^{-6}	5.6	6.6	7.5	11.0
	1 x 10^{-5}	9.7	14.9	15.7	18.5
35°C	1 x 10^{-6}	2.5	2.8	2.8	
	5 x 10^{-6}	11.5	13.2		
	1 x 10^{-5}	22.1	22.9	22.8	
25°C	1 x 10^{-6}	2.1	2.6	3.3	
	5 x 10^{-6}	10.3	11.8	13.6	
	1 x 10^{-5}	18.7		22.9	
0°C	1 x 10^{-6}	1.8	2.1	2.3	3.4
	5 x 10^{-6}	7.8	9.0	9.7	12.9
	1 x 10^{-5}	13.1	17.6	18.3	31.6

TABLE XVII

Percentage of Dye Molecules on Red Blood Cells

Temp.	Initial dye concentration	Time after mixing: 10 minutes	30 minutes	60 minutes	Equilibrium
35°C	1 x 10^{-6}	55.6 %	66.9 %	72.5 %	
	5 x 10^{-6}	50.5	69.4	73.2	
	1 x 10^{-5}	55.8	66.4	69.2	
25.5°C	1 x 10^{-6}	56.3	60.9	65.4	
	5 x 10^{-6}	45.7	57.1	73.9	
	1x 10^{-5}				76.2 %
0°C	1 x 10^{-6}	36.8	36.2	43.4	56.7
	5 x 10^{-6}	35.1	41.7	46.8	69.4
	1 x 10^{-5}	30.6	40.6	49.2	58.2
35°C	1 x 10^{-6}	65.4	72.2	72.2	
	5 x 10^{-6}	58.7	70.8		
	1 x 10^{-5}	58.0	60.3	60.0	
25°C	1 x 10^{-6}	54.7	68.8	77.3	
	5 x 10^{-6}	49.4	61.7	71.5	
	1 x 10^{-5}	49 5		60.4	
0°C	1 x 10^{-6}	47.2	54.0	61.6	
	5 x 10^{-6}	41.0	47.7	50.8	
	1 x 10^{-5}	35.0	46.3	48.1	
25°C	1 x 10^{-6}	55.0	64.4	68.7	81.8
	5 x 10^{-6}	46.6	63.0	75.0	67.8
	1 x 10^{-5}	46.7	61.2	68.7	57.5
Averages					
35°C		57.2	67.1	71.7	
25.5°C		50.5	62.4	70.0	76.2
0°C		37.6	44.4	50.0	65.2

Figure 6 shows the dye on the cells plotted against the total dye in the solution for three different times. These are not selected values; any other data from Tables VII, VIII, or IX will give the same straight lines.

Figure 7, for the reverse reaction, the dye was removed from the cells by a fresh solution. It shows that by approaching the equilibrium from that side we get the same result as in the forward reaction.

Figure 8 shows concentration of dye on cells-time curves. Initial concentrations and temperatures are given for each curve.

Figure 9 gives a summation of all results for 25° C. and 60 minutes after mixing. It includes the results for the changing cell concentrations and the reversal reaction.

Figure 10 illustrates the method used to calculate $\frac{dc}{dt}$ from the time-concentration of dye on the cells curve. Values used are from Table XIII.

Figure 11 shows method used for determining $\frac{dc}{dt}$ from the time-percentage of dye on cells curve. The average percentage values are used as these reflect the average of a number of experiments. The values are from Table XVII.

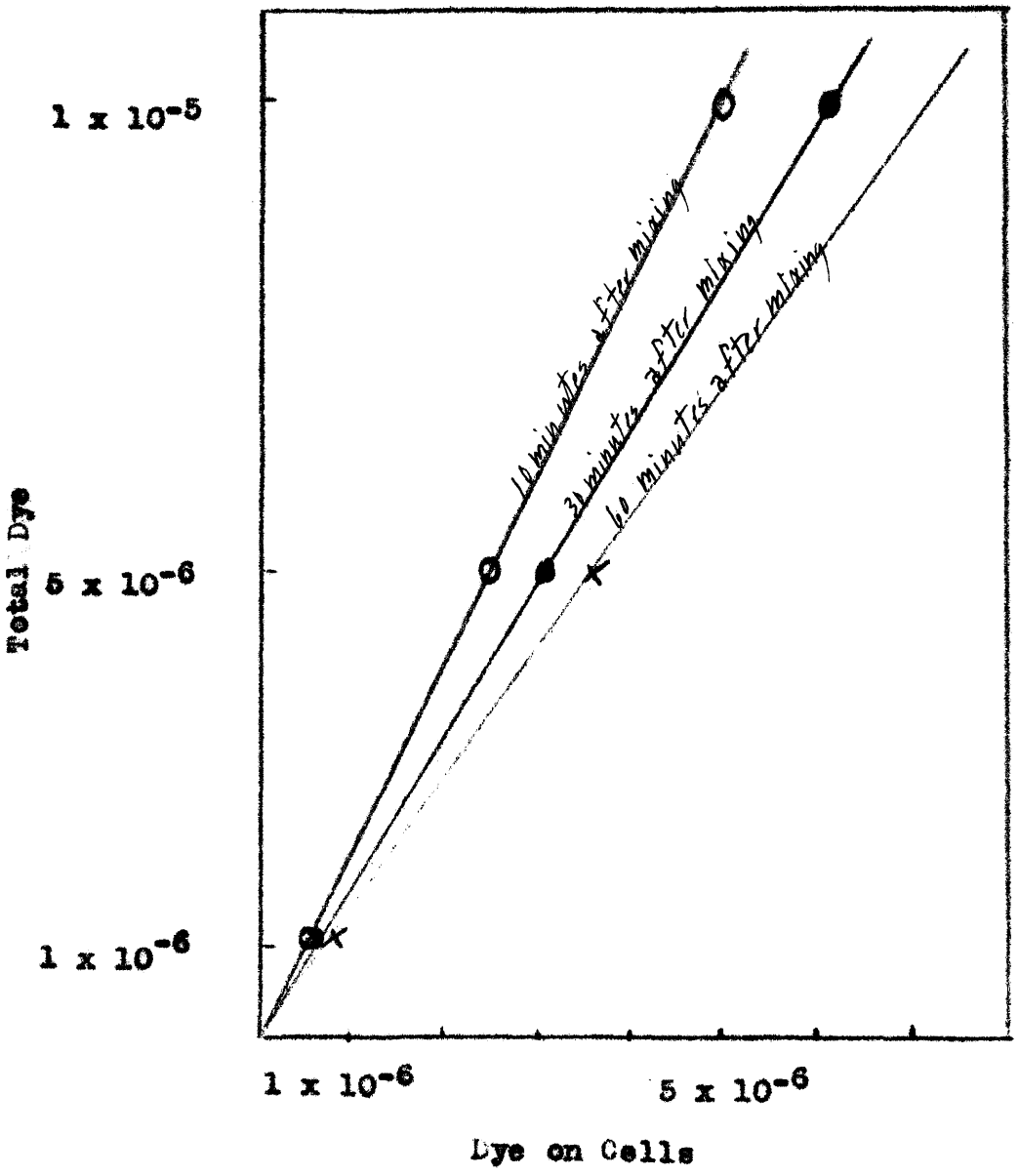
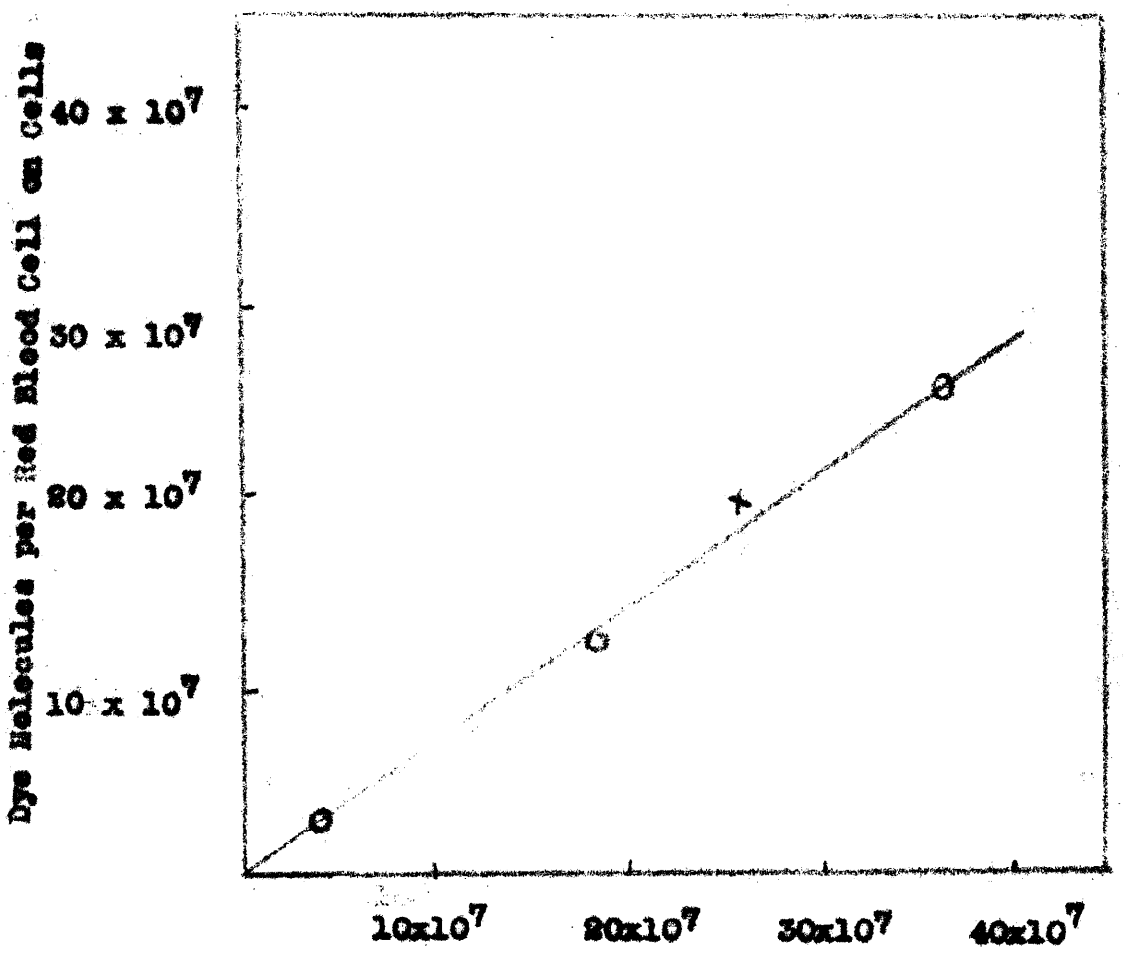

1 x 10⁻⁵
5 x 10⁻⁶
1 x 10⁻⁶
Total Dye
10 minutes after mixing
30 minutes after mixing
60 minutes after mixing
1 x 10⁻⁶
5 x 10⁻⁶
Dye on Cells

Figure 6



Initial Concentration of Dye Molecules per Red Blood Cell

o From equilibrium values Table XIV.

X From reverse reaction Table XIV.

Figure 7

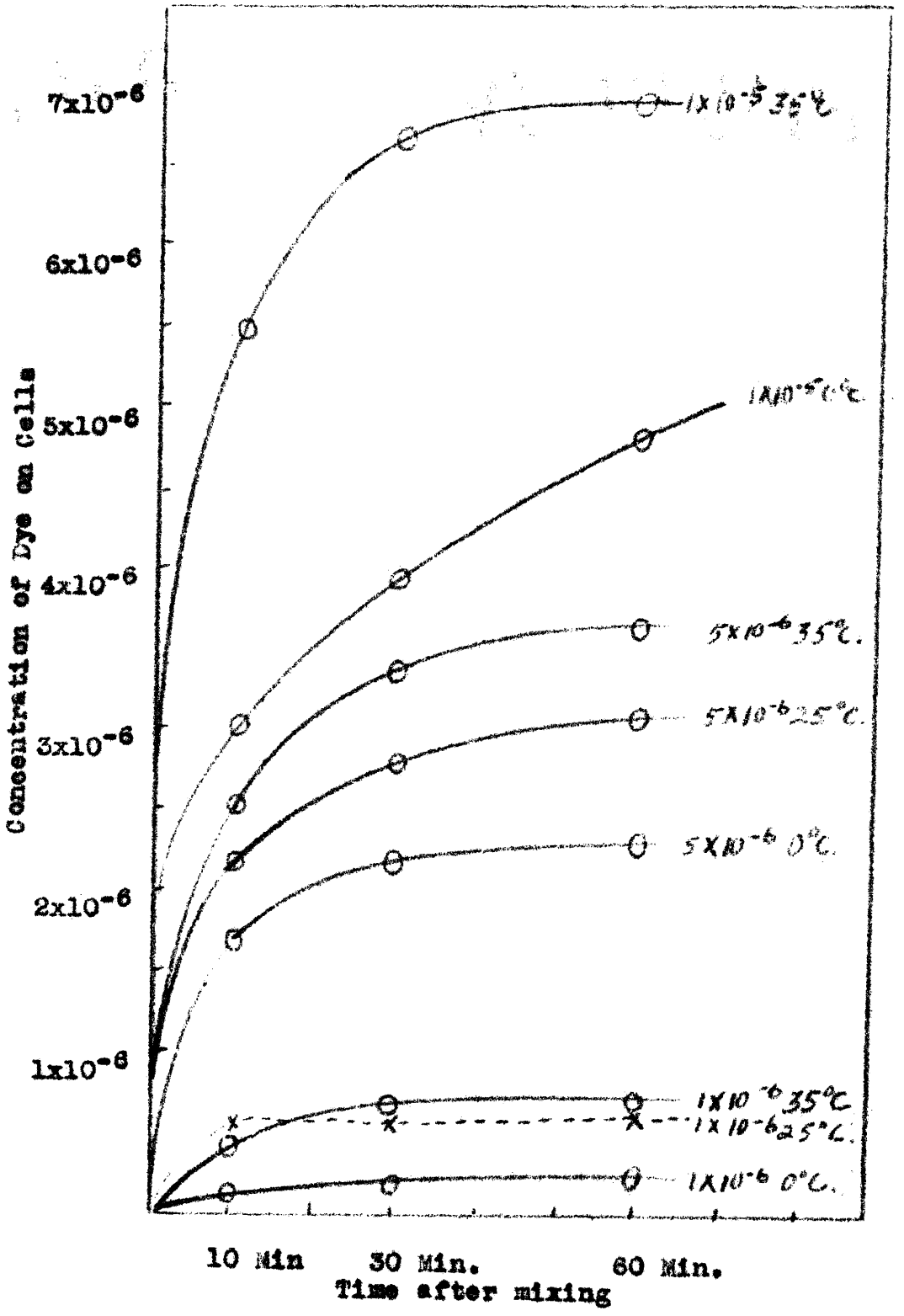

7x10-6
6x10-6
5x10-6
4x10-6
3x10-6
2x10-6
1x10-6
Concentration of Dye on Cells
1X10-5 35°C
1X10-5 0°C.
5X10-6 35°C.
5X10-6 25°C.
5X10-6 0°C.
1X10-6 35°C
1X10-6 25°C.
1X10-6 0°C.
10 Min
30 Min.
60 Min.
Time after mixing

Figure 8

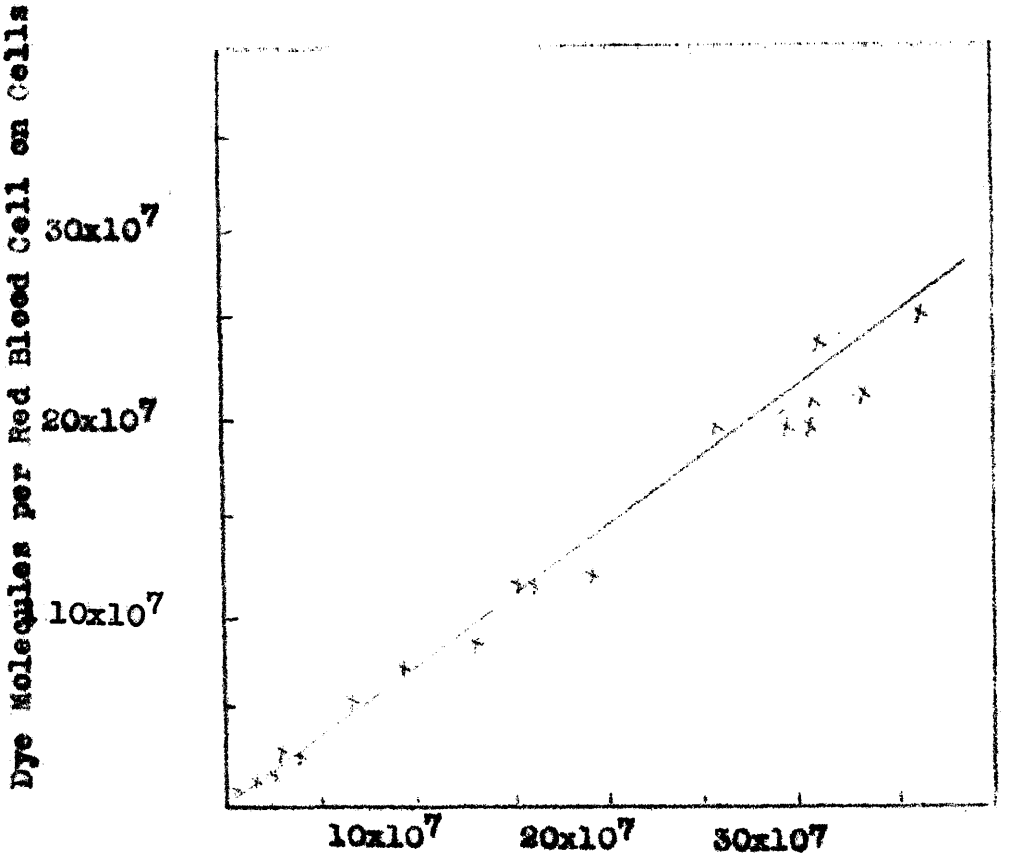

Dye Molecules per Red Blood Cell on Cells
30x10^7
20x10^7
10x10^7
10x10^7
20x10^7
30x10^7
Initial Concentration of Dye Molecules
per Red Blood Cell

Figure 9.

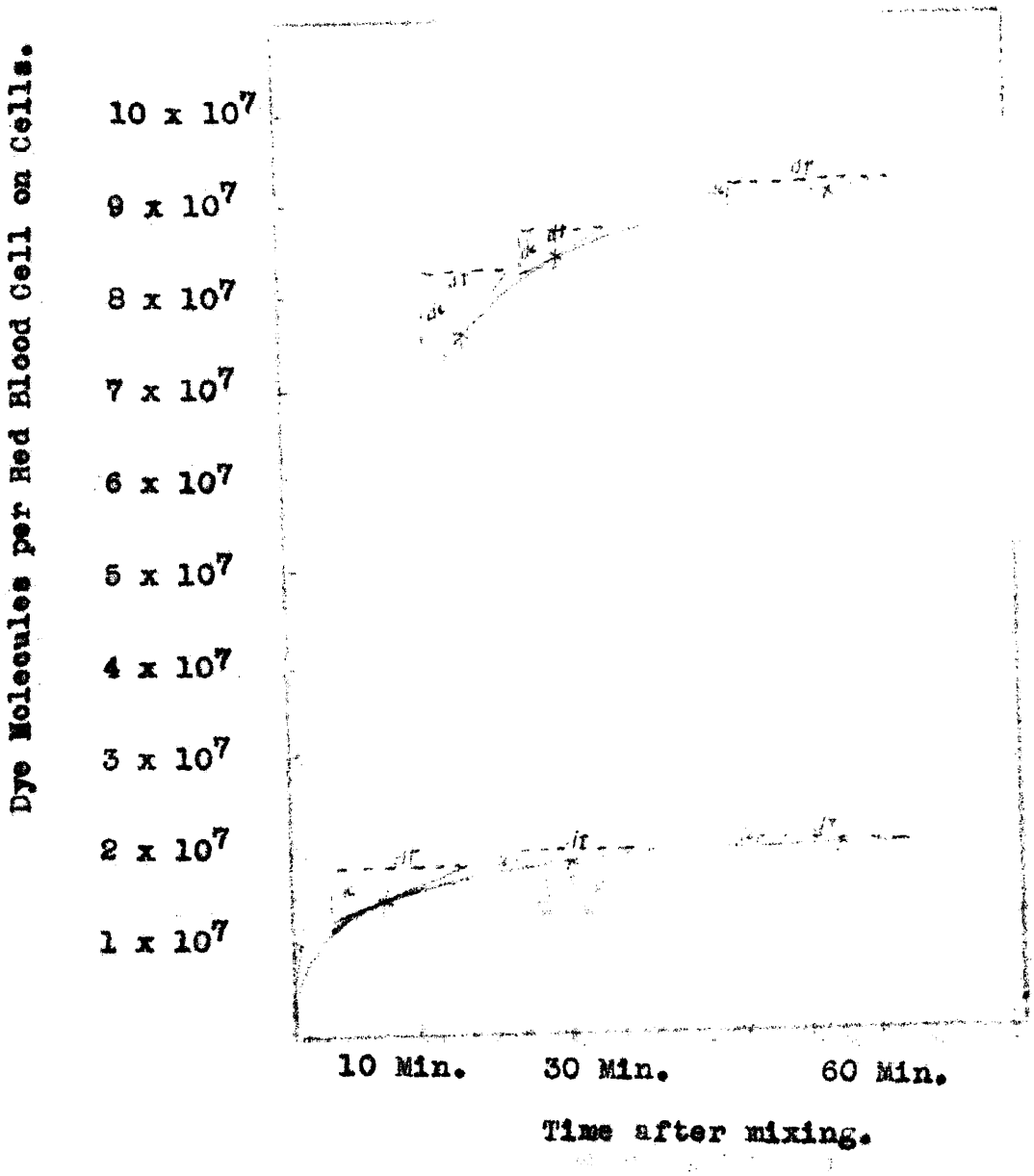

Dye Molecules per Red Blood Cell on Cells.
10 x 10^7
9 x 10^7
8 x 10^7
7 x 10^7
6 x 10^7
5 x 10^7
4 x 10^7
3 x 10^7
2 x 10^7
1 x 10^7
10 Min.
30 Min.
60 Min.
Time after mixing.

Figure 10.

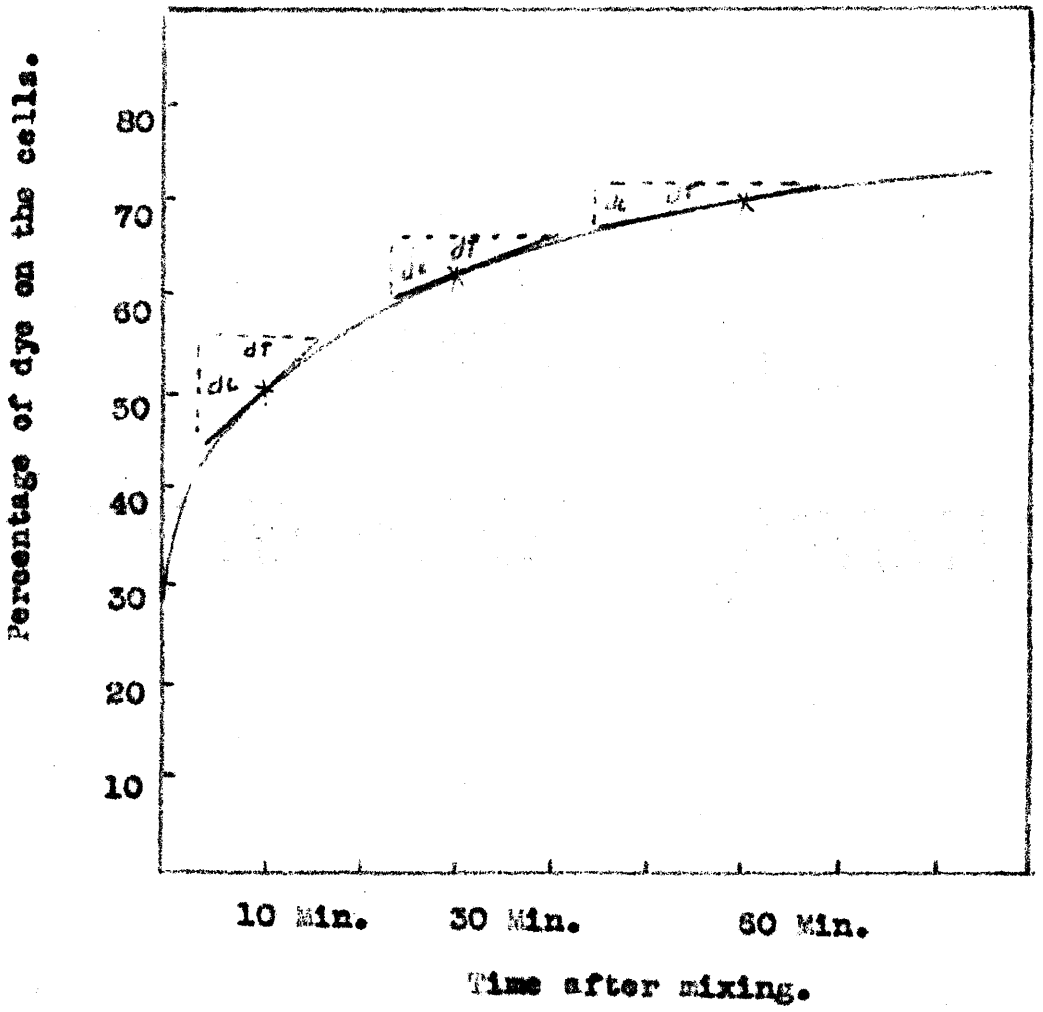

Percentage of dye on the cells.
80
70
60
50
40
30
20
10
10 Min.
30 Min.
60 Min.
Time after mixing.

Figure 11.

DISCUSSION

A great variation was found between the results of this investigation and the results reported by Jodbauer and Haffner.[6] They found equilibrium in ten minutes. In this, equilibrium is not reached until at least three hours. Also they made no correction for colored material from the cells. In these experiments concentrations of cells such as they used were impossible because the amount of colored material from the cells would be far greater than the readings from the dye. However, they used beef cells while this investigation was performed on rabbit cells.

When the amount of dye on the cells is plotted with the initial concentration of the dye, each time interval gives a straight line, Figure 6. A calculation of the percentage of surface covered assuming that the dye is adsorbed on the outer surface of the cells shows a variation of surface coverage from about two per cent to twenty two per cent. Table XVI. A straight line relationship could not be expected with such a wide variation in the surface coverage.

There is a possibility that the dye is being adsorbed on a much larger surface. The cell membrane is a layered structure containing lipide molecules with long paraffine chains oriented perpendicular to the surface and with protein leaflets oriented with the long axis parallel to the surface.[8] This would present a large surface for adsorbing the dye molecules and could give a linear relationship over the range of concentrations used. It is, however, difficult to picture

the large rose bengal molecule adsorbed on the individual protein and lipide chains.

The removal of the dye from solution has a positive temperature coefficient. More dye is removed from solution at the higher temperature than at the lower temperature. As a rule adsorption has a negative temperature coefficient. Less is removed from solution at the higher temperature than at the lower temperature. The removal of the dye from solution would have to be in some other manner than adsorption.

Another possibility would be the formation of a loose compound with the protein, lipide or both. By assuming that one molecule of dye reacts with one molecule of the reacting substance in the cell membrane it is possible to calculate the amount of substance in the membrane which reacts with the dye (see Calculations, Part 6, page 23). The kinetic equation for a bimolecular reaction

$$\frac{dc}{dt} = K_1(a-c)(b-c)-K_2c$$

fits within a single dye concentration. Thus from the rate of reaction, if it is a chemical reaction, it must be a bimolecular reaction. This gives the absurd conclusion that the concentration of the substance in the cell membrane available for the reaction varies with the concentration of the dye, 13.8×10^{-7} molecules per red blood cell for 5×10^{-6} initial dye concentration and 2.87×10^{7} molecules per cell for 1×10^{-6} dye concentration. This would be

predicted by the fact that the concentration on the cells at any time is proportional to the concentration in solution.

Any other order of reaction would give

$$\frac{dc}{dt} = k_1(a-c)^m(b-c)^n - k_2 c^l$$

where m and n are the number of molecules of dye and substance in cell wall which react to form l molecules of compound. It is obvious that no values other than 1 for m, n and l will give the same rate of reaction.

It is possible that there are two reactions occurring at the same time, one between the lipide molecules and dye and the other between the protein molecules and dye. These two reactions may balance in such a manner as to give a direct proportionality between the dye on the cells and the dye in solution. This is a remote possibility for one temperature. It is hardly probable that such a balance could be maintained for different temperatures.

The substance which reacts with the dye may be assumed to be in such a large quantity as to be very little changed by a ten-fold change in dye concentration reacting. Thus in the equilibrium

$$\frac{c^l_{\text{dye.b compound}}}{c_b^m \times c^n_{\text{dye}}} = K$$

the concentration, c_b^m, is assumed to be such a large value that it is practically constant.

$$\frac{c^{l}\ \text{dye b (compound)}}{c^{n}\ \text{dye}} = \text{a constant}$$

For this reaction the dye on the cells is directly proportional to the dye in solution only in the case where n and l are equal to 1. This is a bimolecular reaction which has been shown to be impossible.

It is necessary therefore, to assume that the reaction is between the dye and both components of the membrane to explain the rates of reaction. Blum and Gilbert[2] have calculated, by assuming a molecular weight of 100,000 for the protein molecule and 500 for the lipide molecule, that there are about 10^7 molecules of protein and 10^9 molecules of lipide in the membrane of the cell. To obtain a value for the reacting part of the membrane, with a value large enough that its reacting concentration is very little changed by a reaction in which between 2×10^7 and 25×10^7 molecules of dye react, one must assume that a number of dye molecules are reacting with each molecule of protein. Also the last molecule to react must react as readily as the first one. This is hardly possible with such a large molecule as rose bengal.

From Figure 7, the reaction is over ninety per cent reversible and from Figure 8, the amount taken up by the cell is proportional to the amount in solution per cell. Table XVII shows that for a given temperature the percentage of dye molecules on the cells is practically the same for

any one time. The best explanation of this would be that the dye is distributed between two solvents with the membrane as one solvent and the buffer as the other. A calculation of the concentration of the dye in the cell membrane would give a 2.7×10^{-1} molal solution being extracted from a 1×10^{-5} solution of dye in water. This is a 10,000 times concentration in the cell membrane, but this may be explained by the fact that the membrane is probably a rather thick jelly structure which would tend to hold the molecules of rose bengal in its structure. This would also explain the slow rate of reaction.

A calculation of the reaction velocity based on the assumption of a monomolecular condensation and evaporation from the cell membrane indicates that there are probably two reactions. The pseudo constants are not constant.

$K_1 = 0.0324$ for ten minutes,
$= 0.022$ for thirty minutes and
$= 0.009$ for sixty minutes.

(See Calculations, page 25). This would indicate that there are two components of the cell membrane removing dye from solution. There are two structures proposed for the red cell membrane.[7,8] One is a structure of lipide molecules in one continuous layer with protein molecules in another layer. This structure would give the dye being distributed between the first layer and the water solution and between the first and second layer. This would give as the kinetic equations

for the reaction between the solution and the first part of the membrane

$$\frac{dc}{dt} = K_1(a-c_1-c_2)-K_2c_1$$

and between the two parts of the membrane

$$\frac{dc'}{dt} = K_3(c_1-c_2)-K_4c_2$$

The other structure is a mosaic of lipide and protein. The two components would each remove dye from solution directly. The kinetic equation for this would be

$$\frac{dc}{dt} = K_1(a-c_1-c_2) + K_2(a-c_1-c_2) - K_3c_1 - K_4c_2$$

It is not possible to calculate the individual velocities of these reactions without some knowledge of the concentrations of the dye in each part of the cell c_1 and c_2. At the present time there seems to be no method for determining these values.

The best explanation for the removal of the dye from solution by rose bengal is that the dye is distributed between immiscible solvents, the protein and lipide of the cell membrane and the water solution.

Such a large concentration of dye in the membrane should affect its structure. The cells are normally discoids. The dye causes some change in shape. The discs gradually lose their shape and finally become spheres without an appreciable change in volume with increasing dye concentrations. Higher concentrations of dye will cause hemolysis of the cells but this concentration is an order of magnitude higher than the concentrations used in these experiments.

SUMMARY

Time concentration values for temperatures of 35° C., 25° C., and 0° C. have been determined for the removal of rose bengal from aqueous solution by red blood cells. The reaction is not adsorption or the formation of a chemical compound. The best explanation is that it is a distribution of dye between the water solution and both the lipide and protein fractions of the cell membrane.

BIBLIOGRAPHY

1. Blum, Harold F. and Howard W. Gilbert
Studies of Photodynamic Hemolysis with Monochromatic Light. The Reciprocity Law.
Journal of Cellular and Comparative Physiology
Volume 15, No. 1, page 75. (1940)

2. Blum, Harold F. and Howard W. Gilbert
Quantum Requirements for Photodynamic Hemolysis.
Journal of Cellular and Comparative Physiology
Volume 15, No. 1, page 85. (1940)

3. Blum, Harold F., N. Pace, and R. L. Garrett
Photodynamic Hemolysis I. The Effect of Concentration and Temperature.
Journal of Cellular and Comparative Physiology.
Volume 13, page 269. (1937)

4. Daniels, Farrington. Chemical Kinetics. Ithaca
Cornell University Press (1938)

5. Getman, Frederick H., and Farrington Daniels
Outlines of Theoretical Chemistry, New York
John Wiley and Sons, page 87, (1931)

6. Jodlbauer, A., and F. Haffner
Uber die Wirkung von Eosin und Rose Bengal auf rote Blutkorperchen und den Zusammenhang von Aufnahme und Biolischer Wirkung, Pflugers Archiv fur die Gesamte Physiologie, 189: 234-260 (1940).

7. Ponder, Eric. The Mammalian Red Cell and the Properties of Hemolytic Systems. (Berlin: Bontraeger 1934), Protoplasma Monographien Sechster Band.

8. Schmitt, Francis O. The Ultrastructure of Protoplasmic Constituents. Physiological Reviews, Volume 19, No. 2, Page 296. (1939)

9. Taylor, Hugh S. A Treatise on Physical Chemistry, New York. D. Van Nostrand. (1931)

10. Taylor, Hugh S. Elementary Physical Chemistry. New York. D Van Nostrand (1927)

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N. L. Drake and H. W. Gilbert.
Some Gamma-Nitro-Beta-furylbutyrophenones.
Journal of the American Chemical Society,
52, 4965. December, 1930.

H. F. Blum and H. W. Gilbert.
Quantum Requirements for Photodynamic Hemolysis.
Journal of Cellular and Comparative Physiology.
Volume 15, No. 1, February 1940.

H. F. Blum and H. W. Gilbert.
Studies of Photodynamic Hemolysis with
Monochromatic Light. The Reciprocity Law.
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Volume 15, No. 1, February, 1940.

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