SULFHYDRYL GROUPS AND THE INTERACTION BETWEEN THE HEMES IN HEMOGLOBIN

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I

INTRODUCTION

As early as 1903 Christian Bohr demonstrated that the oxygenation of mammalian hemoglobin follows an S-shaped curve (fraction oxygenated *vs.* oxygen pressure), and does not follow the simple hyperbola predicted by mass-action considerations for the reaction $Hb+O_2 \rightleftharpoons HbO_2$.

Through the work of Adair, Pauling, Wyman, and others this reaction has come to be interpreted in the following way. A molecule of mammalian hemoglobin combines reversibly with 4 molecules of oxygen. The existence of an S-shaped curve is taken to mean that there must be facilitating interactions between the oxygen-combining centers (hemes). These centers, furthermore, are all identical and are believed to be bound in the same manner to the protein. Thus, when an oxygen molecule combines with one center it greatly increases the likelihood that a second oxygen will be attached to another. This is what is meant by interaction (*cf.* Wyman, 1948).

The mechanism of interaction, however, is far from clear. Two recent suggestions have been made. One of these is that the interaction might have a steric origin. A "loosening" of structure is assumed to occur upon initial oxygenation, thus promoting subsequent oxygenation (Pauling, 1949; St. George and Pauling, 1951). The other suggestion, closely related to the first, is that the interaction is largely an entropy effect associated with a large configurational change in the protein (Wyman and Allen, 1951).

I wish to report in this paper observations which indicate that sulfhydryl (—SH) groups of hemoglobin are closely linked with the mechanism of heme-heme interaction in the oxygenation process. Free —SH groups are known to occur in human hemoglobin (Hughes, 1949; Ingbar and Kass, 1951)—with which the present experiments were performed; and in the hemoglobins of the horse (Mirsky and Anson, 1936) and dog (Benesch; *cf.* Hughes, 1949). Blocking

* This research was supported in part by a grant to Professor George Wald by the Biosciences Division of the Office of Naval Research. I am greatly indebted to Professor Wald for suggesting this work, and for helpful advice during its execution.

the —SH groups of human hemoglobin causes a large decrease in interaction between the hemes; this inhibition is lifted by the addition of glutathione. In addition, dialysis against a slightly alkaline buffer results in a loss of interaction, which also is reversed by the addition of glutathione. No such loss of interaction occurs when the hemoglobin is stored without dialysis at the same pH and temperature.

II

Preparation and Methods

The entire preparation of hemoglobin is carried out at 4–7°C. Freshly drawn citrated or oxalated human blood[1] is washed three times with 0.9 per cent NaCl, and the packed cells are laked by the addition of an equal volume of distilled water. After 1 ½ to 2 hours, the laked cells are centrifuged. To the clear supernatant is added an equal volume of pH 8.68 borate buffer (0.03 M $Na_2B_4O_7$, 0.08 M H_3BO_3). This buffered solution (final pH about 8.5, ionic strength about 0.24) is used for the determination of the oxygen equilibrium curve of fresh material. Dialysis is carried out as follows: 5 to 8 ml. of the buffered solution is dialyzed against 2 liters of the borate buffer (pH 8.68), diluted 1:1 with distilled water.

The oxygen equilibrium was determined by the spectrophotometric method and apparatus described in a previous paper (Riggs, 1951). The wave length, 460 mμ, was used for the determination of all oxygen equilibrium curves. The entire absorption spectra of the completely reduced and oxygenated preparations were measured between 460 and 620 mμ in all experiments. Equilibration was carried out at 20°C. by rotation of the tonometer in a water bath at 60 R.P.M. for 20 minutes. Tests were made in each experiment at low, medium, and high oxygen saturations to determine whether this period of equilibration was adequate under the varied experimental conditions. It was found to be so except at oxygen saturations greater than about 80 to 85 per cent; here the solutions were equilibrated for 30 to 40 minutes, producing an increase in saturation up to ½ to 1 per cent more than that obtained with a 20 minute equilibration. The oxygen pressures calculated have been corrected in all experiments for the oxygen combined with the hemoglobin.

III

Effects of Dialysis

The data may be examined in the following way. A simple measure of the inflection of an oxygen equilibrium curve (per cent oxygenation y *vs.* oxygen pressure p) is furnished by the empirical constant, n, in Hill's equation, $\frac{y}{100} = \frac{Kp^n}{1 + Kp^n}$. The constant K is a measure of the affinity for oxygen. When n is one there is no heme-heme interaction, and the y *vs.* p plot is hyperbolic.

[1] I am very grateful to the Mt. Auburn Hospital, Cambridge, for the donation of many of the blood samples used in these experiments.

When n is more than one there is interaction between at least two hemes such that the oxygenation of one is closely associated with the oxygenation of the other; the y *vs.* p plot is S-shaped.

A simple way of calculating n is to transform Hill's equation into the logarithmic form, $\log \frac{y}{100-y} = \log K + n \log p$. The plot, $\log \frac{y}{100-y}$ *vs.* log

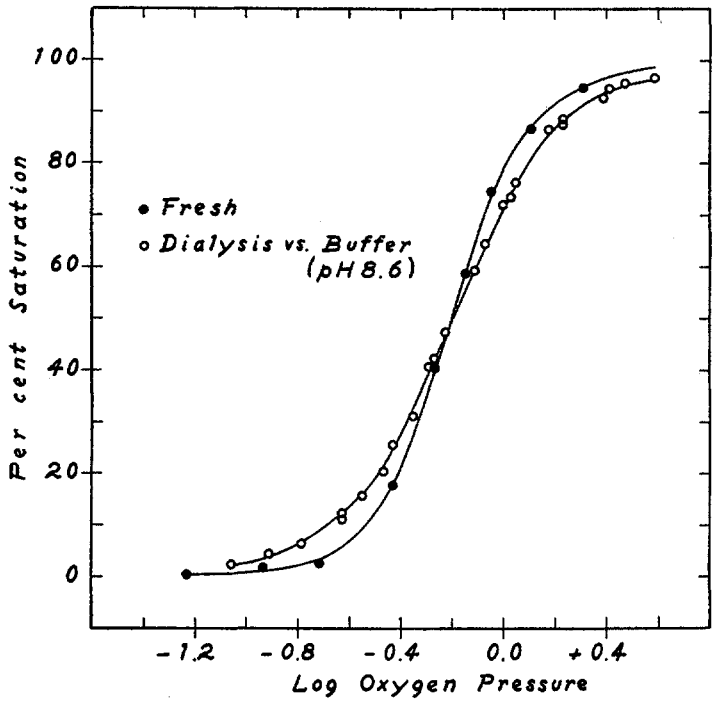

FIG. 1. The effect of dialysis against borate buffer, pH 8.68, upon the oxygen equilibrium of human hemoglobin. Curves for both fresh and dialyzed solutions were determined in borate buffer, pH 8.5, 20°C. It is clear that the slope of the curve decreases upon dialysis. Both sets of data have been moved along the log p axis to coincide at 50 per cent saturation.

p, is a straight line with slope n and intercept K. Throughout this paper n is evaluated with the aid of this linear relation in the neighborhood of 50 to 60 per cent oxygenation. The oxygen equilibrium curves, however, are plotted as y *vs.* log p. In this type of plot all curves which obey Hill's equation and possess the same value of n have the same shape, and can be superimposed by simple transposition along the log p axis. This shift corresponds to differences in K. In this way of plotting the data the slope at the midpoint is directly proportional to n.

Fresh human hemoglobin yields an oxygen equilibrium curve exactly superimposable upon the normal curve reported by Allen, Guthe, and Wyman (1950), with $n = 2.9 \pm 0.1$. After dialysis against pH 8.68 borate buffer, human hemoglobin yields curves for which $n = 2.0 \pm 0.1$. The p_{50} (oxygen pressure for half saturation of the hemoglobin) decreases from 1 mm. Hg to 0.6 to 0.7

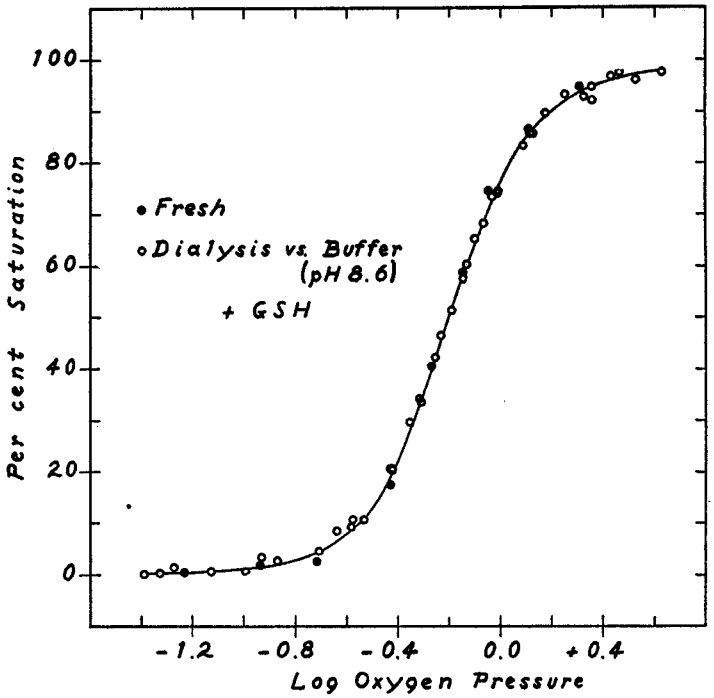

FIG. 2. Glutathione reverses the effect of dialysis upon the oxygen equilibrium of human hemoglobin. Buffered glutathione (1.2 to 4.0 $\times$ 10^{-2} M) has been added to the dialyzed solution. All the data have been adjusted to coincide at 50 per cent saturation.

mm. Hg. On adding glutathione to such dialyzed hemoglobin, n rises again to about 2.5 to 2.6, and the p_{50} increases to about 0.8 mm. Hg. Storage at the same pH and temperature without dialysis has no significant effect upon n or p_{50}. It is true that after such storage the hemoglobin possesses a somewhat higher affinity for oxygen at low oxygen saturations than normally. At higher saturations, however, the oxygen equilibrium curve is indistinguishable from that obtained with fresh hemoglobin (Fig. 3). That is, the normally symmetrical equilibrium curve becomes asymmetrical on storage. This departure from symmetry confirms the observation of Allen, Guthe, and Wyman (1950) that

old hemoglobin preparations give asymmetric curves while fresh preparations do not.

These data are plotted in Figs. 1, 2, and 3 from the values presented in Tables I and II. Inasmuch as the major interest in this paper centers on the shapes of the curves, the data in the graphs have frequently been shifted along the log p axis to coincide at 50 per cent oxygenation.

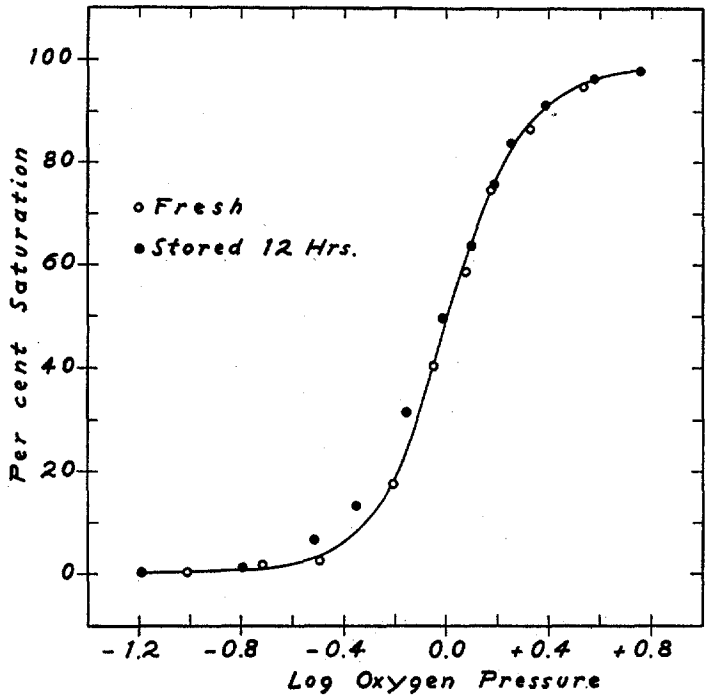

FIG. 3. The effect of storage at 4°C., pH 8.5, for 12 hours. Both curves determined in borate buffer, pH 8.5. No adjustment of the data has been made.

The glutathione added to the dialyzed hemoglobin solutions was buffered with borate and adjusted to pH 8.5 with a drop of NaOH just prior to the addition. Control experiments indicate that these changes, by themselves, have no significant effect upon the oxygen equilibrium under the existing conditions. The ratio, moles glutathione to moles hemoglobin, was varied between 1.4 and 5.8. The molar concentration of hemoglobin was determined by measuring the oxygen capacity, 4 moles of oxygen being equivalent to 1 mole of hemoglobin.

In all these experiments the absorption spectra of both reduced and oxygenated solutions were measured between 460 and 620 mμ. These are all identical

except in the range 600 to 620 mμ; the absorption of oxyhemoglobin rises slightly at 620 mμ in the presence of glutathione and after storage.

TABLE I

The Oxygen Equilibria of Human Hemoglobin at pH 8.4–8.5 in Borate Buffer, 20°C., under Various Conditions

p_{O_2} is the oxygen pressure in millimeters of Hg; y is the per cent oxygenation; p_{50} is the p_{O_2} at 50 per cent saturation.

	Fresh		Dialysis *vs.* borate buffer						Storage for 12 hrs.	
			Experiment 1		Experiment 2		Experiment 3			
O_2 capacity..	1.60×10^{-3}M		3.32×10^{-3}M		3.79×10^{-3}M		3.52×10^{-3}M		3.24×10^{-3}M	
p_{50}	1.06		0.76		0.63		0.56		1.00	
n.....	2.9		2.0		2.0		2.0		2.9	
	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y
	0.098	0.19	0.147	4.27	0.087	2.12	0.250	15.6	0.0653	0.147
	0.193	1.90	0.285	11.0	0.165	6.37	0.454	40.8	0.160	1.17
	0.322	2.53	0.413	20.4	0.236	12.2	0.685	59.7	0.304	6.75
	0.621	17.7	0.534	31.1	0.370	25.5	0.950	73.5	0.444	13.2
	0.904	40.5	0.656	42.1	0.596	47.3	1.53	87.5	0.701	31.7
	1.20	58.8	0.920	59.2	0.840	64.5	2.16	92.7	0.97	49.6
	1.50	74.7	1.20	72.0	1.11	76.2	3.45	96.4	1.24	63.9
	2.13	86.8	1.79	86.6	1.70	88.6			1.52	75.7
	3.42	94.9	5.06	97.0	2.96	95.5			1.81	83.9
									2.44	91.2
									3.74	96.3
									5.72	97.7

IV

Effects of p-Chloromercuribenzoate

Initial experiments with *p*-chloromercuribenzoate (PCMB) were carried out on fresh, undialyzed solutions of human hemoglobin. The addition of PCMB produces a large decrease in the steepness of the oxygen equilibrium curve (y *vs.* log p). In terms of Hill's equation, n drops from 2.9 to about 1.5 to 1.6. The ratio, moles PCMB per mole hemoglobin was varied between 2.0 and 8.8 without significant change in the shape of the curve. Paralleling the decrease in n, the p_{50} shows an increase from 1 to as high as 2.3 mm. Hg. Considerable variation, however, was observed in the p_{50}, particularly at higher concentrations of PCMB (3×10^{-3} M). The data suggest that we may be dealing with two effects. The first appears at low concentrations of PCMB, with a large drop in n, and a relatively small change in p_{50}; the second effect appears at higher PCMB concentrations, and corresponds to little further change in n, but a large increase in p_{50}.

Dialysis for 12 hours before adding PCMB has the effect of magnifying the drop in n. Fig. 4 shows a curve obtained after such a dialysis (data given in Table III). A buffered solution of PCMB was added to the dialyzed hemoglobin in such proportions that the final pH and buffer concentrations were the same as those in previous experiments. The ratio, moles PCMB per mole heme, was 1.0. This curve is symmetrical and $n = 1.4$. Dialysis alone results in a decrease

TABLE II

The Oxygen Equilibria of Dialyzed Human Hemoglobin in the Presence of Various amounts of Glutathione

p_{O_2} is the oxygen pressure in millimeters of Hg, p_{50} is the oxygen pressure at 50 per cent saturation; y is the per cent oxygenation. Concentrations are in moles per liter $\times 10^3$.

	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5	
O_2 capacity	1.94		2.68		2.04		2.48		1.88	
Glutathione	40.7		27.6		21.1		13.7		11.9	
Moles GSH / Moles hemoglobin	5.75		2.57		2.60		1.38		1.58	
pH	8.51		8.58		8.48		8.53		8.48	
n	2.56		2.44		2.50		2.57		2.50	
p_{50}	1.02		0.85		0.72		0.79		0.69	
	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y	p_{O_2}	y
	0.0658	0.0	0.064	0.37	0.061	1.43	0.095	0.80	0.289	10.9
	0.163	0.51	0.160	1.83	0.155	2.87	0.186	3.20	0.531	34.2
	0.318	4.60	0.309	8.44	0.301	9.10	0.466	20.8	0.771	58.1
	0.470	10.7	0.592	29.7	0.433	20.6	0.730	46.4	1.05	74.1
	0.611	20.4	0.873	51.3	0.562	33.5	1.00	65.1	1.64	89.7
	0.890	42.3	1.17	68.2	0.825	57.4	1.60	86.1	2.93	96.8
	1.18	60.2	1.78	85.8	1.11	74.6	2.87	94.9		
	1.48	73.5	3.08	92.0	1.41	83.2				
	2.10	85.7	5.68	97.5	2.03	93.3				
	3.40	92.9			3.31	97.6				
	5.37	96.0								

in both n and p_{50}. Addition of PCMB results in a further decrease in n, but an increase in p_{50} to the original value for fresh hemoglobin.

The sharp drop in n produced by PCMB is in large part reversed by the addition of glutathione (GSH). PCMB was added to a dialyzed solution of hemoglobin, and 2 hours were allowed to elapse before the addition of GSH. The reversal of the PCMB effect is illustrated in Figs. 5 and 6 from the data given in Table III. The increase in p_{50} observed in this experiment is probably due partly to inadequate buffering of the glutathione; the pH dropped 0.4 unit when the GSH was added.

The spectra of both reduced and oxygenated hemoglobin in the presence of PCMB have been measured between 460 and 620 mμ, and do not display any

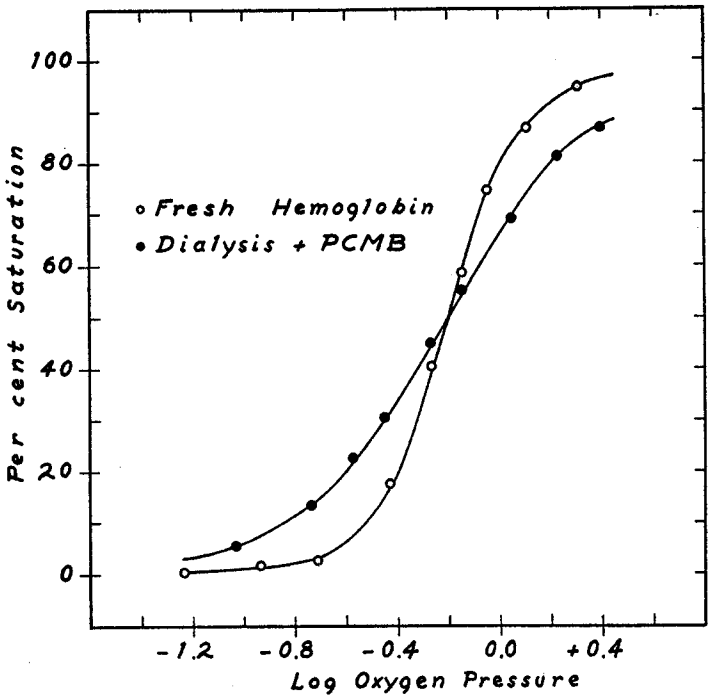

FIG. 4. The effect of dialysis plus the effect of *p*-chloromercuribenzoate upon the oxygen equilibrium of human hemoglobin. Dialysis against borate buffer, pH 8.68. Equilibria determined in borate buffer, pH 8.5, 20°C. The data have been adjusted to coincide at 50 per cent saturation.

TABLE III

Oxygen Equilibria of Human Hemoglobin in the Presence of p-chloromercuribenzoate (PCMB) and p-Chloromercuribenzoate plus Glutathione (GSH)

Both preparations dialyzed. Concentrations of GSH and PCMB in moles per liter × 10^3. 20°C.

GSH	22.2		None	
PCMB	1.60		1.82	
O_2 capacity	1.40		1.83	
p_{50}	1.48		1.01	
n	2.64		1.40	
	p_{O_2}	y	p_{O_2}	y
	0.324	2.20	0.150	6.3
	0.629	9.64	0.299	13.1
	0.918	22.2	0.439	22.6
	1.195	38.5	0.584	30.4
	1.78	60.7	0.880	45.0
	2.38	79.3	1.18	55.0
	3.66	90.4	1.82	69.0
			2.79	81.1
			4.11	86.9

significant alteration. The oxygen capacity also is not changed in the presence of PCMB.

Preliminary measurements[2] of the sedimentation constant of fresh oxyhemoglobin in the presence of PCMB do not indicate any splitting of the molecule such as is observed in the presence of urea (Steinhardt, 1938).

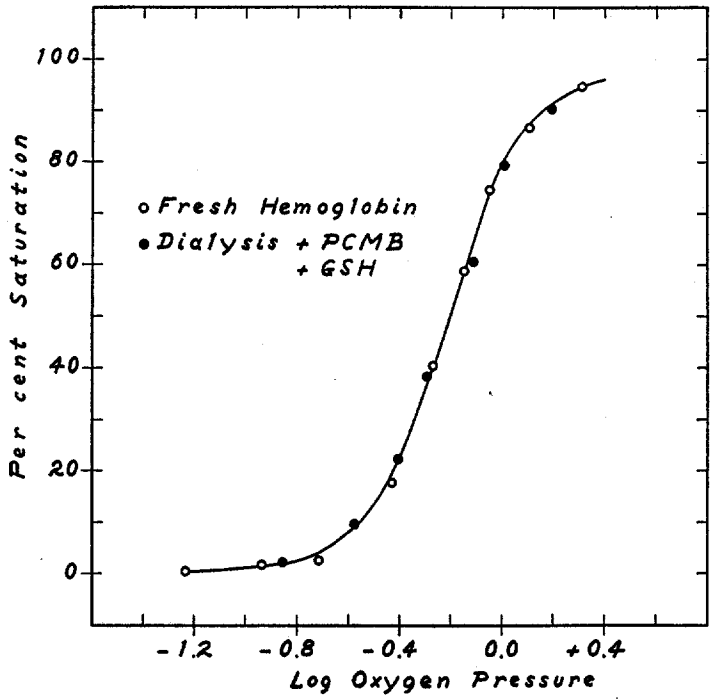

FIG. 5. The reversal with glutathione of the effect of dialysis and *p*-chloromercuribenzoate upon the oxygen equilibrium of human hemoglobin. The data have been adjusted to coincide at the point of 50 per cent saturation.

I have assumed that *p*-chloromercuribenzoate, at least in very low concentrations, is specific for the —SH groups of hemoglobin (Hellerman *et al.*, 1941). It is possible to produce almost the entire change in interaction described here by adding just 2 moles of the mercurial per mole of hemoglobin. This is consistent with the estimations of Hughes (1949) and Ingbar and Kass (1951) that native human hemoglobin contains 2 —SH groups available for titration by CH_3Hg^+ and Ag^+ ions.

[2] These measurements were carried out by Professor J. L. Oncley of the Laboratory of Physical Chemistry related to Medicine and Public Health, Harvard University, to whom I am very grateful.

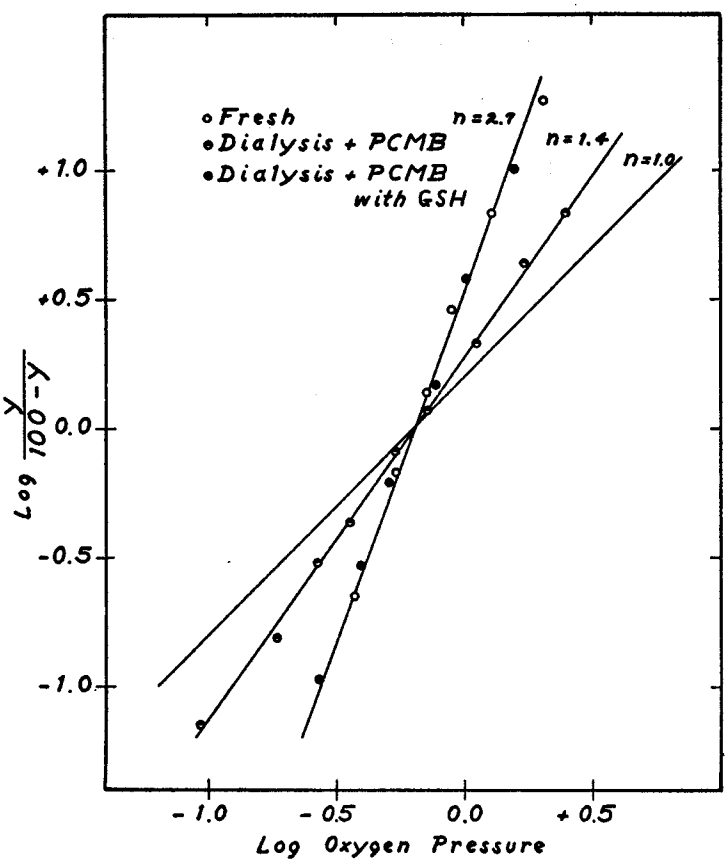

FIG. 6. The effect of *p*-chloromercuribenzoate upon the oxygen equilibrium of human hemoglobin and its reversal with glutathione. The data have been adjusted to coincide at 50 per cent saturation.

V

DISCUSSION

The preceding observations show that the —SH groups of hemoglobin are closely associated with the mechanism of heme-heme interaction. When these groups are blocked with *p*-chloromercuribenzoate (PCMB) or are removed indirectly through some effect of dialysis, the interaction as measured by n in Hill's equation greatly decreases. So far as I know, the only previous observation implying that —SH groups might be involved in the interaction is that of Hill and Wolvekamp (1936), who found that glutathione appeared to make the oxygen equilibrium curve of a dilute hemoglobin solution more highly inflected.

Sulfhydryl groups are known to occur in a number of mammalian hemo-

globins. Horse hemoglobin (Mirsky and Anson, 1936) and normal human hemoglobin (Hughes, 1949; Ingbar and Kass, 1951) have two such groups per molecule. Human sickle cell hemoglobin apparently contains three —SH groups (Ingbar and Kass, 1951). Benesch (*cf.* Hughes, 1949) has reported finding four —SH groups in dog hemoglobin.[3] On titration with silver ions in 20 per cent methanol, the —SH content of both normal and sickle cell human hemoglobin increases to 4 to 5 groups per molecule (Ingbar and Kass, 1951). Greenstein (1939) found a similar increase in the —SH titration of horse hemoglobin denatured with urea or guanidine.

The data on the effects of PCMB on the oxygen equilibrium of hemoglobin recall results obtained by quite different means by other workers. For example, Altschul and Hogness (1939) prepared hemoglobin by prolonged dialysis against distilled water and then passed their solution through aluminum hydroxide. The shapes of the oxygen equilibrium curves they obtained are highly pH-dependent: n varies from about 2.6 at pH 6.3 to about 1.3 at pH 8.6. Similarly, Guthe and Wyman (1952)[4] have described experiments on the oxygen equilibrium of hemoglobin in the presence of formaldehyde with very similar results. I have plotted their data in Fig. 7 using their most alkaline preparation. The data of Altschul and Hogness are similar but exhibit a much greater scatter; for this reason I have omitted them from the figure. A striking similarity exists in the results obtained by dialysis and adsorption, by treatment with formaldehyde, and PCMB. It should be noted here that formaldehyde, like PCMB, is an excellent —SH reagent (Schubert, 1936; Ratner and Clarke, 1937).

There is, however, an important difference between the results obtained in the presence of formaldehyde and in the presence of PCMB. Formaldehyde lowers both n and p_{50}, whereas PCMB lowers n and increases p_{50}. This discrepancy suggests that, while both reagents bind —SH groups, other groups also may be involved. It is important that the concentration of formaldehyde used to produce the effect was 50 to 100 times as great as that of PCMB, and that formaldehyde of course binds —NH_2 groups as well as —SH groups.

The experiments indicate that dialysis at pH 8.5, by itself, greatly reduces the interaction between the hemes, although not to the same extent as PCMB. This effect is reversed with glutathione. Ferrihemoglobin (methemoglobin) does not appear to be involved. The oxygen equilibrium curves obtained after dialysis are symmetrical; the presence of ferrihemoglobin may be expected

[3] As I have just learned from Dr. Benesch, this titration was performed in 33 per cent ethyl alcohol. It is probable that, as in human hemoglobin, this kind of treatment increases the number of available —SH groups.

[4] I am greatly indebted to Drs. Guthe and Wyman for allowing me to use these data prior to publication.

to produce asymmetry (see Allen, Guthe, and Wyman, 1950). Dialysis also does not appear to alter the absorption spectrum.

This suggests that dialysis results in an attack upon certain —SH groups of hemoglobin essential to the normal interaction between the hemes, and that this effect is reversed by the addition of glutathione. It is quite possible that the traces of heavy metal ions in the buffer against which the hemoglobin was

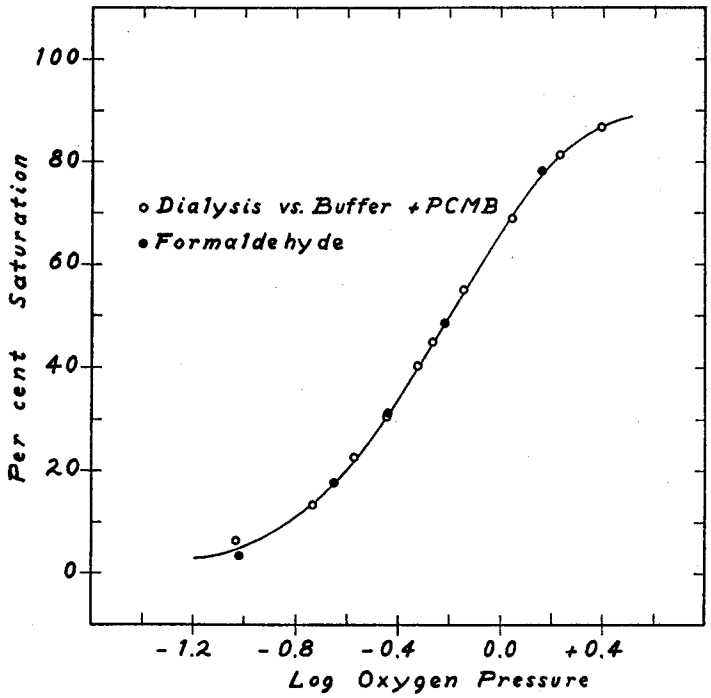

FIG. 7. Comparison of the effects of *p*-chloromercuribenzoate and of formaldehyde upon the oxygen equilibrium of human hemoglobin. The formaldehyde data are from Guthe and Wyman (1951–52). Both sets of data have been adjusted to coincide at the point of 50 per cent saturation.

dialyzed combine with its —SH groups, or catalyze their oxidation to —S—S—. It is significant that Mirsky and Anson (1936) found that cystine can oxidize all the —SH groups of hemoglobin without forming ferric iron. They found that the —SH titration is increased in alkaline solution, and that this increase is reversible, for it is abolished by bringing the pH back to neutrality. It should be noted that —SH groups are more sensitive to oxidation in alkaline solution.

Very similar results were obtained with horse hemoglobin in concentrated urea (~4 M) by Wyman (1948). The oxygen equilibria for the two situations are compared in Fig. 8. The shapes of the two curves are identical, and the

increase in oxygen affinity ($1/p_{50}$) is roughly similar in both sets of data. Wyman obtained a ratio, p_{50} native/p_{50} urea, of about 2.75; the ratio here, p_{50} native/p_{50} dialyzed, is 1.4, 1.7, and 1.9 for three experiments. The larger increase in affinity obtained in urea is possibly associated with the fact that such high molarities of urea are known to split many hemoglobins into half molecules. There is no evidence that such splitting occurs as a result of dialysis. Never-

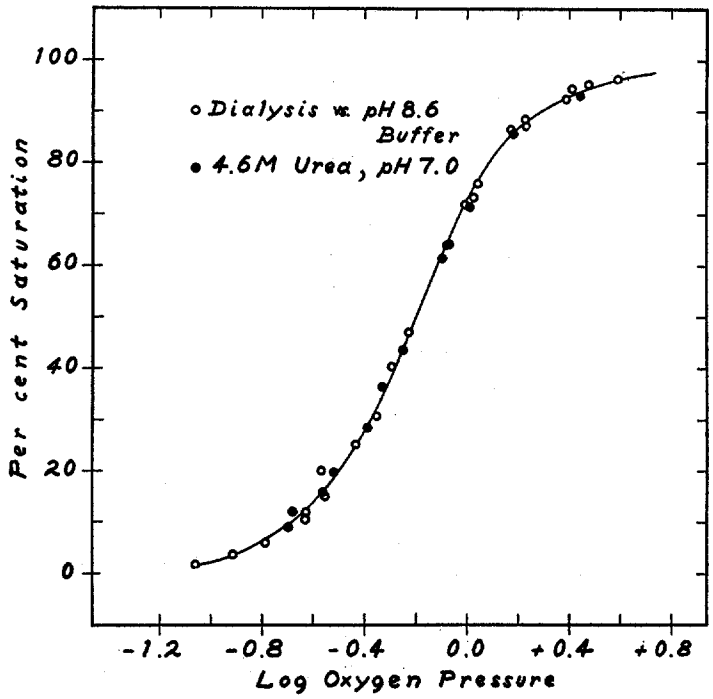

FIG. 8. Comparison of the effects of dialysis against borate buffer (pH 8.68), and of treatment with 4.6 M urea (Wyman, 1948). Both sets of data have been adjusted to coincide at the point of 50 per cent saturation.

theless it seems possible that the results obtained by Wyman (1948) in urea, and those obtained here by alkaline dialysis might be explained by a common mechanism. It may well be significant that both in urea (Greenstein, 1939) and in alkaline solution (Mirsky and Anson, 1936) there is an increase in the sulfhydryl titration.

The present experiments on the role of —SH groups in heme-heme interaction have close parallels also with recent experiments on the mechanism of gel formation by serum albumin in the presence of high concentrations of urea (Huggins, Tapley, and Jensen, 1951). Huggins found that gel formation is inhibited by PCMB; it is facilitated by small concentrations of glutathione,

but inhibited by large concentrations. Sulfhydryl groups might participate in gel formation by forming —S—S— bridges. Since serum albumin has only one readily available —SH group (Hughes, 1949), only dimers would appear possible by this mechanism. Huggins therefore suggests a chain reaction whereby the binding of one —SH group would so alter the protein structure by a redistribution of —SH and —S—S— groups that at least one more —SH group is liberated capable of binding another molecule and so starting a chain. This would explain the facilitating effect of low concentrations of glutathione and the inhibition produced by large concentrations.

Very similar results are obtained with sickle cell hemoglobin. When a concentrated solution of sickle cell hemoglobin is reduced, it forms a birefringent gel which is abolished upon the readmission of oxygen (Sherman, 1940; Stetson and Chandler, 1948; Pauling *et al.*, 1949; Harris, 1950; see also Kass *et al.*, 1951; and Ingbar and Kass, 1951). Gel formation is inhibited by PCMB. Addition of glutathione produces rapid and spontaneous sickling of cells, even in air, while dialysis of such preparations renders them incapable of sickling. Perhaps the effect of glutathione is to reduce certain intramolecular —S—S— bridges, thus liberating —SH groups for a chain reaction of the type suggested by Huggins.

The experiments on gel formation of reduced sickle cell hemoglobin and those reported here on heme-heme interaction have striking parallels and suggest the possibility of a common mechanism. PCMB inhibits gel formation by reduced sickle cell hemoglobin and also radically decreases the interaction between the hemes in normal human hemoglobin. Furthermore, glutathione facilitates gel formation in sickle cell hemoglobin and increases heme-heme interaction. It also reverses the effects of PCMB. These observations indicate that —SH groups are involved in both the sickling process and in heme-heme interaction. The mechanism by which they play a part in these processes is not yet clear. It is probable that —SH groups engage in other types of reaction in addition to oxido-reductions; for example, hydrogen bonding, or combination with carbonyl groups. Very little is known as yet about such reactions in proteins.

SUMMARY AND CONCLUSIONS

1. Dialysis of a hemoglobin solution against slightly alkaline buffer (pH 8.68) causes a decrease in heme-heme interaction. The value of n in Hill's equation drops from 2.9 to 2.0, while the oxygen affinity increases slightly. Addition of glutathione largely reverses the effects of dialysis (n rises from 2.0 to 2.5 to 2.6).

2. Addition of the sulfhydryl reagent, *p*-chloromercuribenzoate (1.8×10^{-3} M), results in a large though not complete blocking of heme-heme interaction. The value of n drops from 2.9 to 1.4. This effect is largely reversed with glutathione (n rises to 2.6).

3. It is concluded that sulfhydryl groups of globin play a large part in the mechanism of heme-heme interaction. The relationships between these results and certain other types of observation—the effects of formaldehyde and urea, and certain phenomena connected with sickle cell hemoglobin—are indicated and their significance discussed.

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