Interaction of Mercury with Human Erythrocytes

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ABSTRACT The binding of mercury to red blood cells was measured in terms of Hg^{203} uptake and desorption. The significant features of the binding are: (*a*) rapid achievement of equilibrium (3 to 5 minutes); (*b*) release of a Hg-complexing material from the red cells themselves which distorts the binding curves at low concentrations of metal (2.5×10^{-7} to 5.0×10^{-6} M); (*c*) prevention of binding by cysteine, glutathione, penicillamine, and EDTA but not by imidazole or histidine; (*d*) binding of mercury in amounts up to 7 times the reduced glutathione concentration of the cells before combination with glutathione itself; (*e*) binding primarily to sulfhydryl groups of hemoglobin and to a small number of stromal sulfhydryl groups, but also to other non-sulfhydryl cellular ligands after saturation of the sulfhydryl groups. Associated with the binding is inhibition of glucose uptake, induction of loss of K^+, and decrease in osmotic fragility. These effects increase over the range of concentrations (1×10^{-17} to 1×10^{-15} moles of Hg/RBC) well below those that result in saturation of the cellular binding sites; above 1×10^{-15} moles/RBC, the effects decrease as the cells become saturated.

The effects of mercury on red blood cells have been investigated in some detail. Wilbrandt (1), Joyce, Moore, and Weatherall (2), Vincent and Blackburn (3–5), and Waltner and Csernovszky (6) have studied the alterations in Na^+ and K^+ permeability; LeFevre (7), the effects on permeability to glucose and glycerol; Jandl and Simmons (8) the agglutinating action and Jung (9) the effects on osmotic fragility and electron microscopic appearance of the cell. All these studies have described the physiological responses in terms of the concentrations of added metal. It is the purpose of the present communication to describe some of the characteristics of the binding of mercury, itself, to the red cell and to relate the amount of metal bound to the red cells to some of the alterations in physiological parameters that have been described previously by other workers.

Mercury in solution, either as an organic mercurial, a complex with chloride ion, or as free divalent mercuric ion, is known to have a marked chemical

affinity for sulfhydryl groups. According to Stricks and Kolthoff (10) the affinity constant of cysteine for mercuric ion, for example, may approach 10^{42}, in an isolated system. Based on this high affinity, when mercuric ion combines with living cells, it may reasonably be expected to combine most firmly with sulfhydryl-containing constituents. The specific aspects of mercury-binding which were studied included the rate of uptake by the red blood cells, the characteristics of the binding curve itself, the effect of soluble thiol-containing agents on the binding, the distribution of the bound mercury between stroma and intracellular phase, and finally, alterations in cellular reduced glutathione content, induced by mercury.

METHODS

Human blood was collected from hematologically normal adult male and female donors, utilizing heparin as the anticoagulant. After centrifugation, the plasma, white blood cells, and platelets were discarded and the red blood cells washed two times with 0.9 per cent NaCl. Immediately prior to the start of each experiment, the red blood cells were washed a third time with dextran-saline[1] solution and resuspended in the latter. The relative red cell volume of the suspension was adjusted to values between 1 and 40 per cent. The use of dextran-saline rather than NaCl as the suspending medium retarded the colloid osmotic hemolysis which red blood cells undergo after becoming permeable to Na^+ and K^+. In no experiments did more than 1 per cent of the cells hemolyze and in most there was essentially no hemolysis. The characteristics of the binding of mercury to the red cells were no different when the cells were suspended in dextran than when they were suspended in saline, except that, based on ultrafiltration studies, 5 per cent of the mercury in the medium was found to be bound to dextran, and for this an appropriate correction was introduced. All experiments were conducted within 2 hours of collection of the blood.

To 0.5 ml aliquots of the red cell suspension were added 2.0 ml aliquots of $HgCl_2$ solution in dextran-saline, labeled with Hg^{203} ranging in concentration from 1×10^{-6} M to 1×10^{-2} M. It was necessary to prepare fresh solutions daily when using concentrations of metal lower than 1×10^{-4} M and to utilize only silicone-treated glassware[2] to prevent adsorption of mercury onto the glass. The cell suspension and mercury-containing solution were mixed, incubated at room temperature for varying periods of time, and then the red cells were separated by centrifugation. The supernatant was removed and the mercury not bound to the cells was determined by measurement of the radioactivity in a well-type scintillation counter. In the absence of any added buffer, the final pH of the supernatant was found to range from 6.8 to 6.9. After preliminary experiments indicated that the binding of mercury was no different than at pH 7.4, all experiments were conducted in unbuffered dextran-saline to avoid any competition for the mercury by buffering agents.

[1] 6 per cent *w/v* dextran in saline from Abbott Laboratories, North Chicago, Illinois or Baxter Laboratories, Inc., Morton Grove, Illinois.
[2] Dow Corning 200 fluid (viscosity grade 350 centistokes).

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Measurements of K^+ were made utilizing a Beckman DU flame photometer. Osmotic fragility determinations were performed according to the method of Parpart *et al.* (11). Red blood cell counts were performed with the Coulter electronic particle counter as described by Brecher, Schneiderman, and Williams (12). Reduced glutathione was determined by the method of Grunert and Phillips (13) as modified by Beutler (14). Mercurimetric amperometric titrations were carried out with a rotating platinum electrode as described by Kolthoff *et al.* (15). Hematocrit values were measured by the microhematocrit technique. Hemoglobin was measured by the method of Crosby, Munn, and Furth (16).

In experiments designed to compare the binding of mercury with inhibition of glucose uptake, 50 per cent cell suspensions were incubated for 1½ minutes with

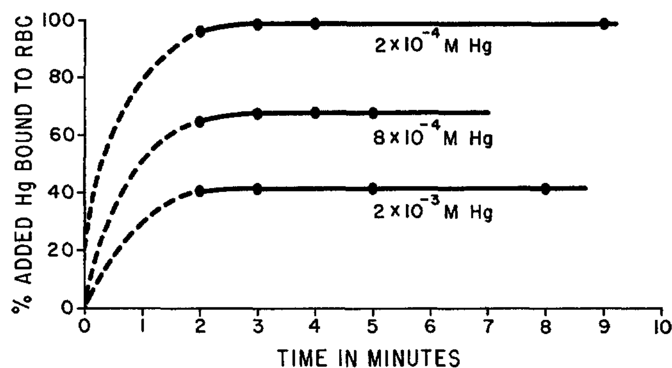

FIGURE 1. Time course of mercury uptake by erythrocytes. Experiments were conducted at room temperature, using a 2 per cent red cell suspension. No measurements were feasible in less than the 2 minutes necessary for centrifugation.

varying metal concentrations. Sufficient glucose was added then to give an initial concentration of 200 mg per cent (in the medium) and the incubation continued for an additional 1½ minutes. The concentrations of glucose in the supernatant were measured by the glucose-oxidase method as described by Saifer and Gerstenfeld (17).

RESULTS

Time Course of Binding and Desorption of Mercury

Over the entire range of mercury and red cell concentrations, the uptake of mercury was found to reach a maximum value in less than 5 minutes (3 minutes of incubation and 2 minutes of centrifugation). Representative data for a tenfold range of spread in concentrations are given in Fig. 1. Once bound, mercury is difficult to wash out of red cells. Five washes with 50 volumes of dextran-saline removed only 6 per cent of the absorbed metal. Five washes with 5×10^{-4} M glutathione (in dextran-saline) removed 14 per cent. Nevertheless, the mercury is reversibly bound, and can be readily exchanged.

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For example, red cells were exposed to 5×10^{-5} M solutions of mercury labeled with Hg^{203}. After 3 minutes the erythrocytes were centrifuged and washed two times. The red cells were then suspended in unlabeled mercuric chloride. At a concentration of 5×10^{-4} M, 18 per cent of the cellular Hg^{203} was lost to the medium in 3 minutes. At a concentration of 2×10^{-3} M non-radioactive mercury, over 75 per cent exchanged in 3 minutes, but little further exchange took place in the next 20 minutes. It can be concluded that the binding curves represent an equilibrium situation, based on the fact that a plateau is achieved within 5 minutes and the fact that the bound mercury is exchangeable.

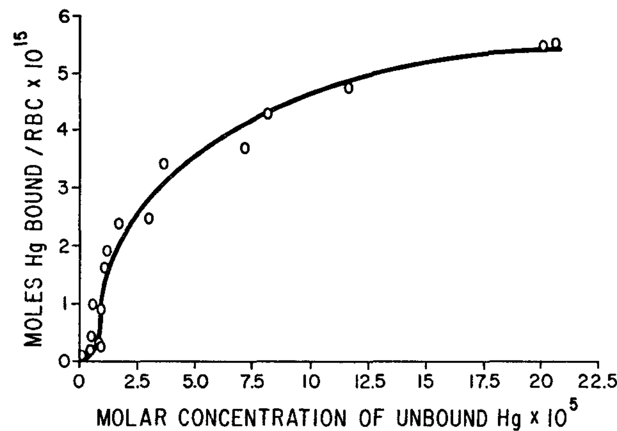

FIGURE 2. Relationship of the mercury bound per red cell to the concentration of unbound mercury. Each point is the mean of duplicate determinations.

Binding Pattern

The relationship of mercury binding to mercury concentration can be represented as a saturation type of curve over a wide range of mercury concentrations (Fig. 2). Although the curve is quite reproducible, detailed examination reveals two deviations from a true asymptotic relationship. One of these is observed at very low concentrations of metal, and one at high concentrations. Fig. 3 is a mass law plot of the data from a representative experiment analogous to the manner in which Scatchard and Black (18) analyzed ion-binding by proteins, and Rothstein and Hayes (19) analyzed ion-binding by cells. Instead of the expected single straight line function based on mass law, Fig. 3 can be described in terms of three segments. The first segment of Fig. 3, representing low amounts of mercury bound to the cells, is anomalous in that the slope is positive. Examination of the metal binding at very low concentrations reveals a distinct toe on the curve (Fig. 2). The size of the toe increased

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markedly with higher concentrations of red cells (Fig. 4). At a hematocrit value of 5 per cent the size of the toe was approximately equivalent to 3 × 10^{-6} M mercury, whereas at a hematocrit value of 50 per cent, it increased to approximately 10 × 10^{-6} M. That the hematocrit reading, *per se*, was not the important factor is shown by the observation that red cells originally prepared at a high hematocrit value (50 per cent) and then resuspended in the same medium but at a low hematocrit value (5 per cent) showed a toe of the same

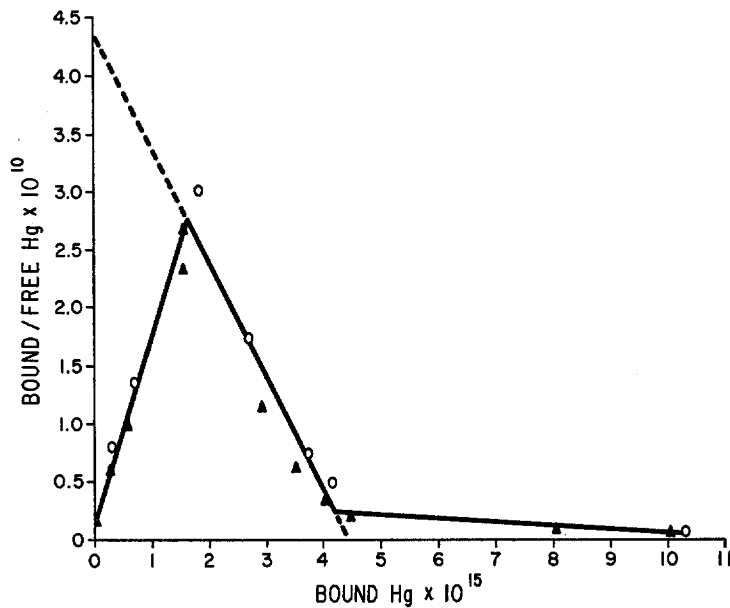

FIGURE 3. Mass law plot of mercury-binding data from two representative experiments. The abscissa is the number of moles of mercury bound per red cell and the ordinate is the ratio of the number of moles of mercury bound per red cell to the concentration of unbound metal.

size as that of the 50 per cent hematocrit value (Fig. 4). Furthermore, the size of the toe increased with the time of incubation of the erythrocytes prior to testing with mercury. Mercurometric amperometric titration (15) of dextran supernatant from a 40 per cent red blood cell suspension revealed the presence of a mercury-complexing material at a concentration of 3 × 10^{-5} M. It can, therefore, be concluded that the red cells, maintained under these conditions *in vitro*, continuously release small amounts of a metal-complexing agent that must be saturated with mercury before appreciable binding of mercury ion to the cells can occur. These observations are in accord with those of LeFevre (7) who found that mercury exerted lesser effects on sugar uptake if the red blood cells were allowed to stand for periods of time before testing.

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Ultrafiltration of the supernatant from cell suspensions revealed that a large part of the complexing agent that leaks out is ultrafilterable and therefore, non-protein in nature. No further effort was made to identify the material.

The middle section of the curve of Fig. 3, exhibiting a negative slope, represents combination of mercury with cellular ligands in accordance with a simple mass law relationship. This relationship may be described as $L +$ Hg $\rightleftharpoons$ LHG, where L is the concentration of ligands, LHg the concentration of bound mercury, and Hg the concentration of unbound mercury, assuming

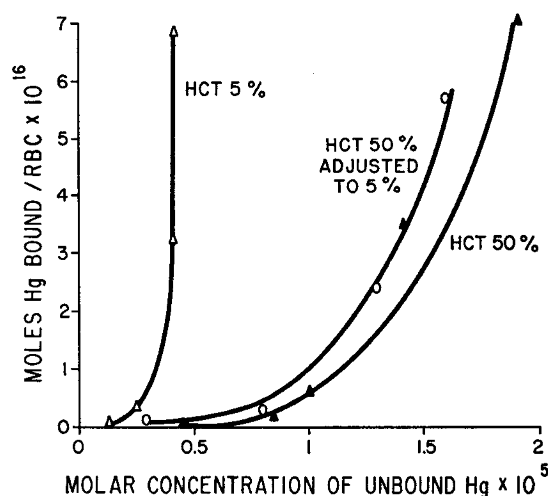

FIGURE 4. Effect of the cell concentration on the binding of mercury. All suspensions were allowed to stand for 2 hours and then either used at that hematocrit value or the value was adjusted prior to use of the sample. △, hematocrit 5 per cent; ▲ hematocrit 50 per cent; ○ hematocrit 50 per cent adjusted to 5 per cent.

no interaction between the binding sites. By extrapolation of the middle segment of Fig. 3 to the x-axis, the maximal number of binding sites can be estimated at 4.2×10^{-15} moles per red cell. In 20 different samples of blood, the mean value was 4.1 ± 0.24 (SD) $\times 10^{-15}$. An affinity constant of 1.03 $\pm$ 0.34 (SD) $\times 10^{5}$ can be calculated from the x and y intercepts[3] of Fig. 3. The affinity constant so calculated is not the association constant of the ligand-mercury complex, because account was not taken of the fact that the mercury in the medium was in the form of a complex ion with chloride or of interaction between binding sites, or of the presence of the complexing material released from the cells. In addition, the affinity constant given for mercury binding to

[3] The equation is the form $B/F = L_T f \cdot K - Bf \cdot K$ where B is bound mercury, F is free mercury, L_T is total binding sites, f is activity coefficient of Hg, and K is the affinity constant. The use of this equation is based on the assumption that each binding site reacts independently of all others.

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red cells is based on the assumption that the ratio of the activity coefficients of the bound and free ligands is 1.0.

The third segment of Fig. 3 at relatively high concentrations of mercury, can be interpreted in terms of binding by another species of cellular ligand with a lower affinity for the metal. A more detailed discussion of such phenomena has been presented by Rothstein and Hayes (19).

Chemical Nature of the Binding Site

The results of competition studies are summarized in Table I. 2.5×10^{-4} M imidazole or histidine failed to alter the pattern of mercury binding in any

TABLE I

MERCURY-BINDING IN PRESENCE OF INHIBITORS

5×10^{-7} moles of each inhibitor was present in each 2.5 ml. of experimental sample. The final hematocrit value was 4 per cent.

	Per cent added mercury bound to RBC						
Moles Added Hg	Control	Imidazole	Histidine	Penicil-lamine	EDTA	Cysteine	GSH
1×10^{-7}	87	87.2	86.1	0	0	0	0
2×10^{-7}	94	92	91.4	0.4	3.6	3.6	0
4×10^{-7}	95.2	96.3	95	10.2	10.2	21.9	12.6
1×10^{-6}	97.3	97.7	97.3	39.6	39.5	52.7	52.8

way. Similar concentrations of either EDTA, reduced glutathione, cysteine, or penicillamine, however, completely prevented binding of mercury, when a molar ratio of inhibition to mercury of 2 to 1 or greater was present. Because only reagents with a high affinity for mercury will prevent binding to the red cells, the cellular sites must also possess a high affinity for this metal. Imidazole or protein carboxyl groups do not satisfy this requirement (20). Evidence which strongly supports the sulfhydryl nature of the cellular binding groups is found in the work of Kolthoff, Stricks, and Morren (15) who demonstrated that mercury, as $HgCl_2$, reacts specifically with sulfhydryl groups in protein molecules in a one-to-one ratio as $(Hg)_2(S)_2$, provided an excess of mercury is present. In fact, mercury has been used as a specific reagent for measurement of sulfhydryl groups of human hemoglobin by Ingram (21), Allison and Cecil (22), and Riggs (23).

Table II represents data obtained from red cells treated with varying amounts of mercury, with measurements of the cellular reduced glutathione content compared with the amount of mercury bound per cell. The table illustrates the point that the mercury which is bound does not react with the cellular glutathione until 2.8×10^{15} moles of mercury are bound per cell

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(70 per cent of the total binding capacity for mercury). Table II also illustrates that 1.9×10^{-15} moles of mercury (7 times the total glutathione content) can be bound to the red cells before the mercury begins to react with the glutathione. This must indicate that other cellular ligands, perhaps sulfhydryl groups of hemoglobin, preferentially combine with mercury and in a sense "protect" reduced glutathione. All the interaction between glutathione and mercury occurs within the middle segment of Fig. 3 and strongly suggests that this portion of the curve represents binding to sulfhydryl groups.

TABLE II

RELATION BETWEEN THE MERCURY BINDING AND THE CELLULAR CONTENT OF REDUCED GLUTATHIONE

The mean corpuscular hemoglobin content of the cells utilized in this experiment was 28.5 $\mu\mu$g. The maximal "sulfhydryl" mercury-binding capacity was 4.1×10^{-15} moles/RBC.

Moles Hg bound/RBC	Moles GSH/RBC	Per cent control GSH
3.8×10^{-16}	2.6×10^{-16}	100
9.5×10^{-16}	2.6×10^{-16}	100
1.9×10^{-15}	2.6×10^{-16}	100
2.8×10^{-15}	6.5×10^{-17}	25
3.0×10^{-15}	2.6×10^{-18}	1
4.1×10^{-15}	0	0

Distribution within the Cell

An estimate of the stromal/non-stromal phase distribution of the bound mercury was obtained by a method depending on centrifugal separation after osmotic hemolysis of the red cells in 50 volumes of distilled water. The details of such a centrifugal separation have been described previously (24). The results are summarized in Table III. At low concentrations of metal, the percentage of cellular mercury bound to stroma was found to be relatively high, 31 per cent for 1×10^{-6} M. However, with increasing concentrations of metal, the percentage bound by stroma was reduced to a few per cent. The maximal binding capacity of stroma, determined by the same method of analysis applied to the data in Fig. 3, is only 5 per cent of the maximal value for the whole cell but in view of the fact that stroma may bind 31 per cent of the mercury taken up at low concentration levels, the affinity of stroma for mercury must be somewhat higher than that of the non-stromal fraction. Analysis of the data on stromal binding by the Scatchard plot indicates that saturation of stromal sulfhydryl groups occurs at a concentration of mercury which saturates only 25 per cent of the total cellular sulfhydryl. This observa-

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tion is consistent with a somewhat higher affinity of stroma for mercury than that of the whole cell for mercury.

In the stromal preparation described above, the hemoglobin content of the stroma is very low, less than 3 per cent of the original cellular content of hemoglobin. On the other hand, in an experiment in which red cell ghosts were prepared by hemolysis in 10 volumes of distilled water, the hemoglobin content was relatively high, 21 per cent of that originally in the cell. These "hemoglobin-rich" ghosts were capable of binding a maximum of 23 per cent

TABLE III

STROMAL-NON-STROMAL DISTRIBUTION OF BOUND MERCURY AT VARIOUS CONCENTRATIONS OF ADDED METAL

2 ml of the mercury solution was added to 0.5 ml of a 4 per cent red cell suspension. Each value represents the mean of at least two determinations. The values given represent the percentage of the total bound mercury found in the stromal fraction.

Concentration added	Moles Hg bound/cell	Hg in stroma
moles/liter		*per cent*
1.0×10^{-6}	6.6×10^{-18}	31
2.5×10^{-6}	1.7×10^{-17}	27
4.0×10^{-6}	2.6×10^{-17}	23
7.2×10^{-6}	4.8×10^{-17}	16
1.0×10^{-5}	6.5×10^{-17}	16
2.0×10^{-5}	1.4×10^{-16}	10
5.3×10^{-5}	3.7×10^{-16}	6
1.0×10^{-4}	7.0×10^{-16}	3
5.0×10^{-4}	1.8×10^{-15}	7

as much mercury as the maximum for the non-hemolyzed red cells from which the ghosts were prepared. When compared with the maximal mercury-binding capacity of the "hemoglobin-poor" ghosts, these results suggest that at high concentrations of mercury, hemoglobin alone or hemoglobin plus other nonstromal constituents is responsible for most of the binding of mercury to cellular sulfhydryl sites.

Loss of K^+ and Inhibition of Glucose Entry

Fig. 5 illustrates the relationship between per cent loss of K^+ and inhibition of glucose entry to the amount of mercury bound per red cell, as studied over a 3 minute interval. The data for both effects follow a pattern that is strikingly similar. The curves reach a maximal value at 1×10^{-15} moles/cell and then show a reversal at higher levels of mercury. The inflection point roughly

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coincides with the concentration of mercury necessary to saturate the stromal sulfhydryl groups (Table II).

Coincident with the reversal of the effects of mercury at higher concentrations, a brownish discoloration of the cellular hemoglobin develops, together with a tendency of the red cells to clump. These observations suggest that a "tanning" or denaturation of cellular protein with a consequent decrease in permeability as described by Wilbrandt (25) had occurred.

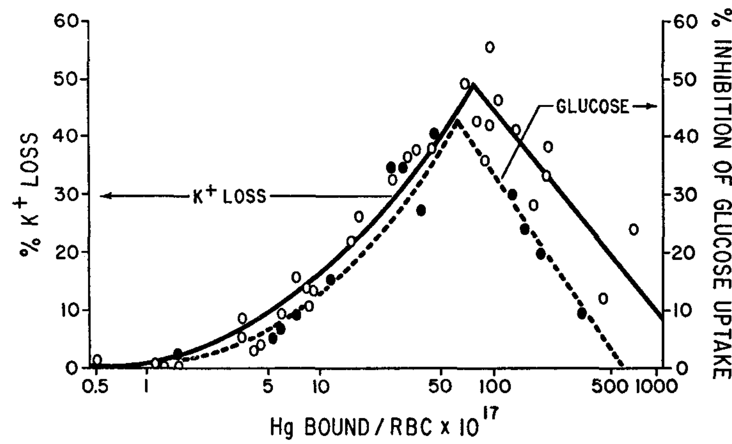

FIGURE 5. Relation of the mercury binding to the loss of K^+ and to the inhibition of glucose entry. The abscissa represents the moles of mercury bound per red cell.

Alterations in Osmotic Fragility

Red cells, after 3 minutes of contact with low concentrations of mercury, manifest decreased osmotic fragility as illustrated in Fig. 6. The stepwise shift of the fragility curve toward the base line, rather than a symmetrical shift of the entire curve to the right, indicates an all-or-none component of the effect of mercury on the red cells with increasing segments of the population becoming resistant to osmotic lysis under the conditions of this experiment. This use of osmotic fragility patterns to describe population behavior has been discussed elsewhere by Eckel (26), Passow and Tillman (27), and Weed, Eber, and Rothstein (28). In contrast, high concentrations of mercury produced a marked increase in osmotic fragility. The pattern of these observations is consistent with Jung's study (9) of mercury-induced alterations in osmotic fragility. The concentrations of metal used in the present study, however, were larger presumably because in the present study, the cells were exposed to mercury for only 3 minutes, whereas the duration of exposure in Jung's study was 6 hours.

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A comparison of Figs. 5 and 6 reveals that the alterations in red cell osmotic fragility correlate well with the effects of mercury on K^+ leakage. The concentrations of mercury which produce a decrease in osmotic fragility are those which produce increasing losses of K^+ from the cells, whereas the high concentrations which induce a decreased osmotic fragility are found also to produce the "tanning effect" (25).

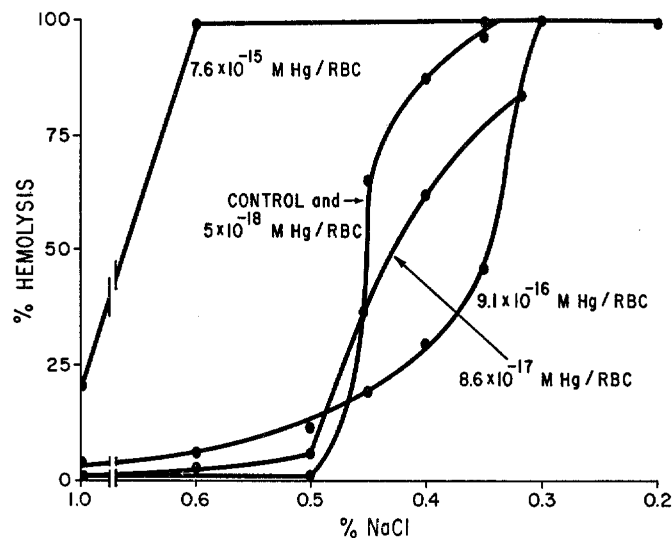

FIGURE 6. Osmotic fragility at different concentrations of bound mercury. Fragility studies were carried out after 3 minutes' exposure of the cells to mercury. A segment of the higher concentrations of NaCl has been omitted for convenience of presentation.

DISCUSSION

In contradistinction to the slow uptake of another divalent ion such as Mn^{++} (24) by the red cell, the uptake of mercury is very fast, with equilibrium rapidly achieved. That this is not indicative merely of surface-binding by stroma is shown by the relatively low capacity of stroma for the metal. In saline solutions mercuric ion exists almost entirely as an anionic complex with chloride. Such an anionic complex might be expected to penetrate red cell membranes with much greater rapidity than a divalent cation.

In attempting to relate the binding of mercury by red cells to the effects of mercury on red cell permeability to K^+ and glucose, as illustrated in Fig. 5, it is apparent that there are functionally at least two types of binding. Decreasing permeability to glucose and increasing permeability to K^+ are produced by concentrations of mercury which have reacted with approximately 25 per cent of the total number of cellular sulfhydryl groups and

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with approximately all the stromal sulfhydryl groups. The binding to the additional 75 per cent of the total sulfhydryl groups in the cell, including reaction with reduced glutathione, results not only in no further increase in K^+ permeability, but actually results, instead, in a decrease. Because the reaction of mercury with reduced glutathione does not occur until 70 per cent of the total cellular sites are occupied by metal, it is suggested that reduced glutathione plays no role in the increasing alterations in permeability produced by mercury.

Passow, Rothstein, and Clarkson (29) have distinguished between cellular ligands whose combination with metals produces an altered permeability and those whose combination with metals has no effect on permeability. The "physiologically non-responsive" binding sites will protect "physiologically responsive" sites by "diverting" the metal. If membrane sulfhydryl groups determine the changes in permeability as suggested by the present study, then the fact that saturation of the membrane groups which constitute 5 per cent or less of the total cellular sulfhydryl groups, does not occur until 25 per cent of the total number of cellular sulfhydryl ligands have reacted with mercury, indicates a protective effect. Apart from permeability changes, this protective effect is also evident in the case of the biochemically important, reduced glutathione which does not combine with mercury until approximately 70 per cent of the total cellular sulfhydryl sites have reacted.

The stable binding of mercury by red cells has been attributed largely to sulfhydryl groups. At least three components which contribute to the total sulfhydryl content have been demonstrated. The stromal groups have the highest affinity but account for a maximum of 5 per cent of the total mercury-binding capacity. The reduced glutathione has the lowest affinity and also constitutes a maximum of 5 per cent of the total. The experiment with hemoglobin-rich stroma suggests that non-stromal protein (presumably hemoglobin), accounts largely for the binding of the remaining 90 per cent of the metal, about 3.7×10^{-15} moles per cell. This value can be expressed as 8.5 moles per mole of hemoglobin, based on the mean corpuscular hemoglobin (28 $\mu\mu$g) of the cells used for this experiment.

Considerable variation exists in published values for the sulfhydryl content of human hemoglobin that has been purified in various ways. The values which have been obtained through indirect measurement with "specific" reagents such as $HgCl_2$ (21–23), *N*-ethylmaleimide (23, 30, 31), 2-nitro-5-chlorobenzoic acid (32, 33), or silver (31, 34, 35) have been found to vary from 2.0 to 8.0 moles sulfhydryl per mole hemoglobin. Cole, Stein, and Moore (31) carried out chromatographic separation of the half-cystine residues of hemoglobin which accounted for 5 sulfhydryl groups per mole of hemoglobin while Braunitzer *et al.* (36–38) have presented evidence that the number of cysteine residues in human hemoglobin is 6. Such chemical analyses indicate

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that the higher values (7.5 to 8.5) for moles of sulfhydryl per mole hemoglobin obtained by the argentometric titration (31, 34, 35) may, in part, represent the measurement of "masked" sulfhydryl groups as opposed to the lower value (approximately 2 SH per mole hemoglobin) obtained by use of *N*-ethylmaleimide (23, 30, 31) which probably represents the readily "reactive" sites. In addition, however, the chemical analyses indicate that silver is bound to groups other than free sulfhydryl groups. In the present experiments the binding of mercury in excess of 6 moles per mole of hemoglobin must also represent binding to groups other than free sulfhydryl groups of hemoglobin. A possible explanation for this excess binding would be formation of some $(Hg)_3(S)_2$ complexes which would not be detectable in amperometric determinations, as pointed out by Kolthoff, Stricks, and Morren (15). In addition, it is possible that there has occurred some reaction of mercury with the sulfur atom in the methionine of hemoglobin, possibly through the catalytic rupture of thio-ether (C-S-C) linkages by mercury (39, 40). The binding of mercury in excess of 6 moles per mole hemoglobin is probably not the result of breakage of disulfide bonds (22, 34, 41), or of binding of mercury to other cellular protein sulfhydryl groups, since Stein *et al.* (42) demonstrated no difference in the titratable sulfhydryl content of hemoglobin purified chromatographically from that purified only by dialysis.

It is of interest to compare the physiological correlation, in the present experiments, of the "reactive" groups in hemoglobin on the one hand, and of the abnormally high values for mercury binding, on the other. The increase in K^+ permeability and decrease of glucose permeability are induced by reaction of mercury with 25 per cent of the total cellular sulfhydryl content, presumably the readily reactive sites. As amounts of mercury are bound which approach the maximal binding capacity and which exceed the total hemoglobin sulfhydryl content, the tanning effect (25) is seen, together with a discoloration of the hemoglobin and a clumping of the cells.

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