about 0.0002*M* (13 ppm) at 300°C to about 0.003*M* (196 ppm) at 500°C. On the whole, precision was poor in the blank runs because of the small weight loss involved; nevertheless they serve to illustrate the orders of magnitude of the background solubilities involved in chloride rich–sulfur poor systems.

On the basis of experimental results established to date on alteration equilibria (*9*), the range in total acidity (*10*) involved in the experiments is not unreasonable. Calculations indicate that the solution *p*H at 300°C on the K-feldspar-muscovite-quartz curve (Fig. 1) is between 4 and 4.5. The degree of base leaching or hydrogen-ion metasomatism, represented by the environment of ore deposition, generally ranges from relatively weak, in the decomposition of Ca-plagioclase to form sericite (muscovite) or montmorillonite, to intense in the alteration of sericite to kaolinite or pyrophyllite. If one assumes even a rather dilute saline environment, an activity of hydrogen ion of about *p*H 5 or less would be required for such processes at elevated temperatures (exceeding 200°C). Solutions whose acidities fall within the neutral-to-moderately basic region of *p*H would not give rise to hydrogen metasomatism, but rather to cation-exchange reactions, feldspathization, and zeolitization.

Although relatively few data points are shown (Fig. 1) for the solubility of galena, one may see that the galena solubilities are essentially the same as those for sphalerite. Because the solubility-product constants for the two sulfides are roughly comparable in this pressure-temperature range, the equivalence of the solubilities suggests that the stabilities of the chloride complexes for both metals are also essentially the same between 300° and 500°C.

The lower-temperature portions of the solubility curves (Fig. 1) are of considerable interest from the standpoint of correlation with theoretical calculations of solubility. Such calculations were made (*11*) for the case in which total metal equals total sulfur under the experimental conditions of this work. At 300°C the calculations have considerable uncertainty but are in close agreement with the solubilities of galena and sphalerite given (Fig. 1). It is interesting that the concentrations of metal-chloride species considered by Helgeson, and found to be most significant under these conditions of moderate acidity, and moderately high sulfur content, are in agreement, within a factor of two, with our experimental results. The present estimates of galena solubility, based on improved thermodynamic data, are higher than those originally computed by Helgeson (*6*). The calculations for 200°C indicate a smooth extension of the solubility curves to lower temperatures, with a greater rate of change of solubility with temperature at lower temperatures.

Below 200°C, the predicted solubility curves for sphalerite and galena in concentrated KCl solutions are essentially parallel. Although the ratio of the solubility-product constants for galena and sphalerite, as a function of temperature, is not constant, the changes in the stabilities of the chloride complexes of lead and zinc with temperature are such that the solubility curves for the two sulfides are parallel in the lower temperature range and approach each other at high temperatures. Calculations of the degrees of formation of zinc and lead chloride complexes (from extrapolated lower-temperature dissociation constants and activity coefficients) indicate that only 0.01 mole percent of the lead and 0.0015 mole percent of the zinc are free in a 2*M* chloride solution at 300°C.

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12. Publication authorized by the director, U.S. Geological Survey.

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Red Cells Coated with Immunoglobulin G: Binding and Sphering by Mononuclear Cells in Man

Abstract. *Human monocytes, macrophages, and certain lymphocytes bind firmly to red cells coated with immunoglobulin G, whether or not it is acting as antibody. Monocyte binding is specific for cells coated with immunoglobulin G and is inhibited specifically by this immunoglobulin or its Fc-fragment in solution. Although not involving serum complement and not usually a prelude to erythrophagocytosis, this binding causes rapid morphological injury to red cells, as manifested by their sphering, increased osmotic fragility, deformation, and fragmentation. It is inferred that mononuclear cells have specific surface receptors for immunoglobulin G and that these provide a critical phase of the mechanism in vivo, whereby red cells or other particles coated with antibody are apprehended and destroyed.*

In man, many antibodies to red cells are very destructive in vivo but cause no hemolysis, agglutination, or evidence of morphologic or metabolic damage to the red cells in vitro. These so-called "incomplete" antibodies are commonly of the 7*S* immunoglobulin G (γG) (*1*) class that fix little or no complement; they are exemplified by the predominant Rh isoantibodies such as anti-D and by most "autoantibodies" of patients with severe forms of immunohemolytic anemia. There is no adequate explanation either for the extraordinary ability of the reticuloendothelial system to trap and destroy red cells coated with these antibodies or for the spherocytosis and increased osmotic fragility that often accompany the hemolytic process.

Red cells coated with incomplete Rh (anti-D) antibodies form rosettes in vitro (*2*) by adhering tenaciously to certain peripheral blood leukocytes. These rosettes were originally observed in saline suspensions of cells, particularly after brief centrifugation, and they occurred with leukocytes from either D-

positive or D-negative donors (*2*); these leukocytes later were identified by Archer (*3*) as monocytes. Subsequently, similar rosettes were observed when peritoneal macrophages from rabbits or guinea pigs were incubated with antibody-coated heterologous red cells, provided complement was inactivated to delay erythrophagocytosis (*4*). It has been assumed that the so-called "cytophilic antibody" described (*4*) is necesary in the initial step in phagocytosis. However, it has not been determined whether the rosettes induced by Rh antibodies are fundamentally analogous to those attributed to cytophilic antibodies or whether either process takes place under physiological conditions. Accordingly, we have reexamined the interaction between antibody-coated human red cells and human peripheral blood leukocytes and tissue macrophages (*5*).

For these studies, leukocytes from dextran-sedimented blood were allowed to settle and attach to the bottom of plastic tissue culture dishes (*6*), the attached cells then forming a monolayer of granulocytes and monocytes that was systematically rinsed free of unattached cells, mainly the lymphocytes. Various preparations of red cells, in some instances labeled with ^{51}Cr, were washed and resuspended at a hematocrit of 3 percent in Hanks's solution. The suspensions were added to the monolayer preparations and incubated for various periods up to 2 hours; after this, unbound red cells were washed free. The leukocytes were then detached from the plates with buffered EDTA (0.2 percent in isotonic phosphate buffer, *p*H 7.4), the suspensions were examined by microscopic and ultramicroscopic techniques, and the quantity of bound radioactive red cells was measured.

When red cells coated with anti-D are incubated with peripheral blood leukocytes in monolayers, the red cells attach to mononuclear cells, creating rosettes similar to those observed in suspensions (*2, 3*). This reaction, taking place from 4° to 37°C, is not dependent on the presence of serum, nor is it inhibited by EDTA. Phagocytosis occurs to only a limited extent, even under optimum conditions in the presence of complement. Red cells coated with γG "autoantibodies" derived from patients with immunohemolytic anemias, whether idiopathic or secondary to the administration of L-3(3,4-dihydroxyphenyl)-2-methylalanine (*7*), form comparable rosettes. In contrast, red cells coated with a variety of γM antibodies, including anti-A, anti-B, and anti-I, are not bound in this system; however, if complement is added to these sensitized cells, a disorderly mixed agglutination occurs, primarily involving granulocytes, and erythrophagocytosis is pronounced (*2*). Red cells that had been injured by prolonged incubation, storage, heating, sulfhydryl blockade, or a variety of metabolic inhibitors were not bound by monocytes. Red cells treated with 1 percent glutaraldehyde and then washed also formed rosette clusters (*8*); these differed, however, in that the clusters were not as orderly or homogeneous and involved many granulocytes and lymphocytes as well as monocytes. Albumin and γG were attached to red cells by means of chromic chloride, one of several multivalent cations that aggregate and couple proteins to red cells by nonimmunological means (*9*). Cells coated in this way with γG reacted very strongly with monocytes, whereas albumin-coated cells and cells treated with chromic chloride alone were unreactive (Table 1.) Red cells heavily coated nonimmunologically with complement (*10*) were also unreactive.

Table 1. Binding by monocytes of red cells coated with various proteins. Under standardized conditions, suspensions of labeled red cells were added to monolayer preparations, and the extent of binding was determined in duplicate for each entire monolayer.

Red cell coating	Agglutination by Coombs serum	Red cells bound $10^5 \times$ No.
None	0	0.4
Rh (anti-D) antibody	4+ (γ)	10.0
Complement	4+ (non-γ)	0.7
Albumin (+ $CrCl_3$)	4+ (non-γ)	0.5
γG globulin (+ $CrCl_3$)	4+ (γ)	29.0
$CrCl_3$	0	0.9

Preparations of human tissue macrophages were obtained from modified Rebuck "skin windows" (*11*) and from spleens obtained surgically from patients with hereditary spherocytosis. In both preparations, a large portion of the macrophages resembled blood monocytes in their ability to form rosettes with red cells coated with anti-D.

Normal serum or plasma inhibited the binding of red cells coated with γG (but not of glutaraldehyde-treated red cells). Of the serum proteins tested, only γG and its Fc-fragment (*12, 13*) were markedly inhibitory. Albumin, γM (*12*), and γA (*12, 14*) caused little or no inhibition at physiological concentrations. Thus it seems that the soluble γG of serum competes with the γG attached to red cells for receptors on the monocyte surface. The inhibitory potency of the Fc-fragment (97 percent inhibition), as compared with the much weaker effect of equivalent amounts of Fab-fragment (31 percent inhibition), suggests that γG is bound by monocytes through binding of its Fc-fragment, a finding analogous to that reported by Berken and Benacerraf (*4*) working with cytophilic antibodies. Inhibition was studied also by varying the proportions of red cells and undiluted serum, the red cells always being in excess of the "binding capacity" of the monolayers. Whereas binding to monocytes was uninfluenced by the concentration of red cells in saline, it increased in direct proportion to the red cell concentration (hematocrit) in serum.

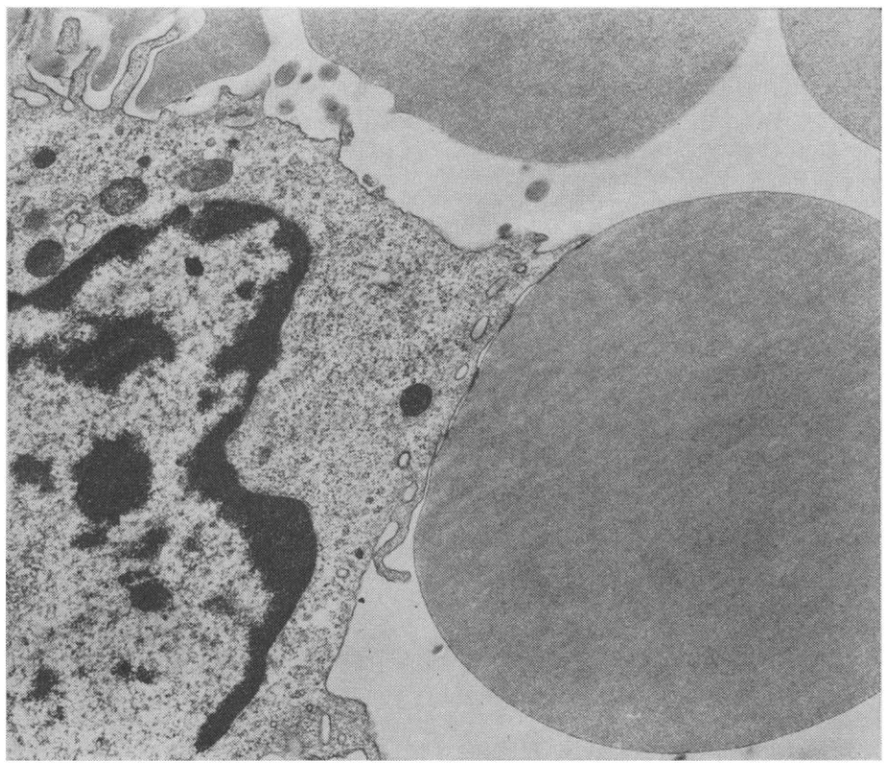
At hematocrit values in excess of 75 percent, comparable to those encountered in the splenic red pulp, serum had little inhibitory effect. The diminished inhibition by serum when the proportion of red cells to serum is increased suggests that entrapment of antibody-coated red cells in vivo is enhanced by plasma skimming and erythroconcentration; the fact that the spleen is notable both for erythroconcentration and for efficient trapping of antibody-coated red cells (*15*) supports this interpretation. The competitive inhibition by serum γG of a binding reaction involving cell-fixed γG is reminiscent of the rheumatoid factors (*16*). However, binding of coated red cells was uninfluenced by Gm (hereditary globulin factor) type and could not be blocked by prior exposure of the monocytes to antiserums reacting with γM or to trypsin. Binding was also unaffected by cyanide and fluoride and was only partially inhibited by dinitrophenol. Sulfhydryl blocking agents prevented, but did not reverse, attachment. Once formed, rosettes resisted disruption, even by prolonged and vigorous mechanical shaking. However, incubation of the rosettes in papain (0.016 g per 100 ml of solution) released almost all of the bound red cells.

Bound to mononuclear cells, antibody-coated red cells rapidly transformed from their normal, flat, disk shape to become spherocytes. Along

with this shape change, the bound red cells became very sensitive to hypotonic lysis, as manifested by a marked, rather symmetrical shift in the osmotic fragility curve. In contrast, those antibody-coated cells, from the same suspension, which did not have the opportunity to adhere to the monocytes remained normal in shape and osmotic fragility. Red cells persisted in their spherical shape and increased osmotic fragility after being detached by papain.

Electron microscopic studies were made of rosettes and control cell mixtures after fixation in 2.5 percent glutaraldehyde in cacodylate buffer, *p*H 7.4, followed by osmium-collidine. The majority of rosette-forming cells of peripheral blood fulfilled all of the ultrastructural criteria for typical monocytes. In some preparations, up to 30 percent resembled lymphocytes. The "monocytes," whether typical or lymphocytoid, gripped the antibody-coated red cells by a variety of projections. On adhering, the cell membranes showed points or plaques of intimate contact which sometimes were regularly spaced (Fig. 1). The monocyte processes varied from long, delicate, finger-like projections, characteristically about 1 μ thick, to processes which grasped the red cell over an extensive area (Fig. 2), sometimes enveloping (phagocytizing) the intact cell entirely. More often, the partly enveloped red cell underwent puckering in the region of attachment, with formation of numerous folds and tubular processes about 1 μ in diameter that sometimes gave an appearance of vacuolation and fragmentation of the red cell (Fig. 2). Often villous processes from the monocyte invaginated deeply into the attached red cell, interdigitating with the red cell processes. Although these red cell deformities and the apparent fragmentation were prevalent, and were presumably responsible in part for the spherocytosis by causing contracture or loss of cell surface (*17*), some bound, spheroidal red cells lacked evidence of irregularity or fragmentation and appeared swollen rather than contracted. The ultamicroscopic appearance of rosettes containing red cells coated with the complexes of γG globulin and Cr^{3+} was similar except for the electron-dense aggregates of Cr^{3+} on the outer surfaces of the red cells.

Our studies indicate that human macrophages, monocytes, and some of the large lymphocytes, but not granulocytes, have unique surface receptors which bind γG. In vivo, these receptors may enable the macrophages under appropriate circumstances to apprehend and trap red cells—and presumably other particles—that are coated with incomplete antibodies. In erythroblastosis fetalis due to the presence of incomplete Rh antibodies, erythrophagocytosis is variable and limited in extent; when seen, however, erythrophagocytosis mainly involves monocytes (*18*). Similarly, phagocytosis of antibody-coated red cells in vitro is rather



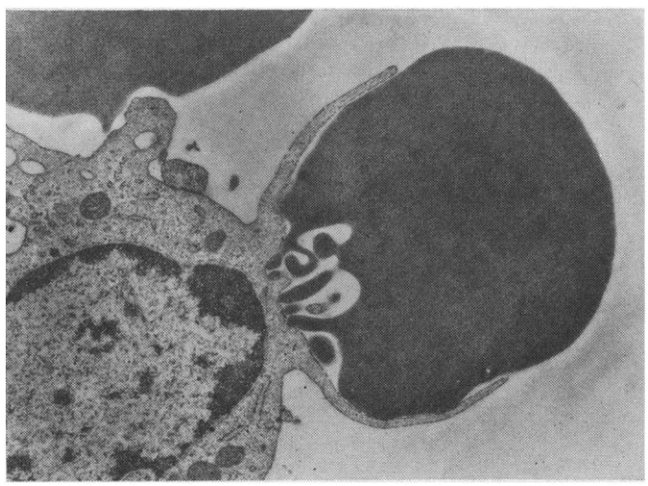


Fig. 1 (top). Sites of attachment of a monocyte to red cells coated with Rh (anti-D) incomplete antibodies. Long finger-like monocyte processes (top left) often invaginate deeply into attached red cells. The smooth spheroidal red cell on the right appears to be attached by means of multiple, regularly spaced points of adhesion. (× 20,000)

Fig. 2 (left). This red cell coated with anti-D is grasped by a cup-shaped process from the monocyte. Extensively bound cells such as this often are smaller and appear darker, presumably as the geometrical consequence of the complex folding of membrane at the attachment site. (× 15,000)

limited and is confined to the monocytes, the principal event being that the red cells are bound tightly to the monocyte surface. This binding of red cells coated with either antibodies or aggregates containing γG does not ordinarily represent merely a preliminary phase of erythrophagocytosis in the usual sense, but it is nevertheless rapidly injurious as indicated by the sphering, deformation, and apparent fragmentation of the bound cells. Although differing in several distinct ways from the cytophilic antibodies described by others (*4*) in animals, these 7*S* γG antibodies of man may represent a more specialized or evolved form of cytophilic antibody in which the function of binding to antigens has been divorced from complement-promoted processes such as phagocytosis and lysis. This would allow an orderly transport of particles to specific tissue sites, and trapping or containment therein, without immediately or necessarily causing phagocytosis, or even lysis, and without the abrupt or dangerous effects of activating complement. The functional adequacy of antibodies that can induce removal of antigenic cells from the blood stream but cannot lyse them or induce extensive phagocytosis is well documented in isoimmune hemolytic processes (*2, 15*). The specific reaction of γG with monocyte and macrophage receptors provides a logical explanation for the mechanism of action of incomplete antibodies in man.

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Electron Microscopy: Enhancement of Specimen Contrast by Injection of Atoms

Abstract. *Injections of biological specimens and substrates with cesium were made with a small accelerator in the energy range of 20 to 1000 electron volts. Subsequent electron microscopic examination demonstrated that the contrast and appearance of the specimen depend on its structure and on the energy of injection. Substrate noise is decreased over conventional contrasting techniques. The same accelerator provides controlled etching of the specimen.*

It is well known that the utility of conventional electron microscopy for investigation of biological specimens, indeed of any material of low atomic number, is very dependent on techniques that enhance contrast. The most common of these are heavy-metal shadowing, negative staining, and positive staining. The first technique outlines the specimen by a surface deposit; the second depends primarily on the drying of a solution of the heavy element around the specimen or in regions of it that are penetrated by the solution; and the third generally involves a more selective chemical interaction between specimen and solution. As instrumental resolution improves, these techniques themselves impose a limit to observable detail. This occurs particularly in the first two because of inherent aggregation of the contrasting atoms. The result is a granularity of at least 5 Å, usually more, providing a noise background in which the specimen must be observed.

Injection of atoms holds promise of improving the signal-to-noise ratio for high-resolution work and of adding a new feature to the examination of specimens in the whole range of electron microscopic observation. In essence, it is an extension of the heavy-metal-shadowing technique. The extension is that of an increase in the energy of the atoms impinging on the specimen by several orders of magnitude.

Consider the appearance of a specimen to an atom speeding toward it with an energy controlled by the experimenter. Even a distant view is that of extremely rough terrain. On closer approach the complicated arrangement of complex molecules with voids and hills appears; still closer, as the atoms of the molecules appear, they are discerned in groups of varied geometrical array as dictated by the chemical bonds between them. Surface and interior voids from one to ten or more bond lengths (a few to tens of angstroms) are seen. There are regions of softness and hardness—weak and strong bonds. Finally there is the inevitable collision of the impinging atom somewhere in this structure—early, if the path encounters a promontory; later, if it coincides with a surface void. If the energy of the approaching atom is low, as in shadowing ($\sim$ 0.1 ev), weak surface forces will be adequate to hold the atom in a surface layer, although not necessarily at the place it strikes. At energies of the order of the bond strength ($\sim$ 1 ev) at the particular point of impact, the atom may be elastically scattered and escape, or it may be captured. Capture can depend on several factors. A sufficiently strong chemical bond, relative to the incoming kinetic energy, may form between the incoming atom and atoms of the specimen. The latter can be displaced, thus