Although we do not know whether PMN leukocytes actually generate $^1O_2$*, their ability to make $\cdot O_2^-$ (*8*), coupled with their unique characteristic of lacking the enzyme superoxide dismutase (*19*), would enable any $\cdot O_2^-$ generated by the metabolic changes induced by phagocytosis to spontaneously undergo dismutation to $^1O_2$*, as described earlier (*7*). These observations raise the question of whether some forms of virulence may be associated with bacterial strains that are particularly effective in neutralizng the lethal effects of $^1O_2$*.

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Mammalian Hepatic Lectin

Abstract. *A rabbit hepatic protein that specifically binds asialoglycoproteins is also a lectin that agglutinates untreated human and rabbit erythrocytes and neuraminidase-treated erythrocytes from rat, mouse, and guinea pig. Both binding of asialoglycoproteins and agglutination of erythrocytes appear to involve reaction on the same active sites of the hepatic protein.*

When the terminal sialic acid residues of their carbohydrate moieties are removed from a number of plasma proteins, the desialylated molecules are rapidly transferred from blood to hepatic parenchymal cells where they are catabolized by lysosomes (*1*). The transfer is initiated by binding of the desialylated protein to a hepatocytic plasma membrane glycoprotein that has been isolated, solubilized, and partly characterized (*2*). We report now on the erythrocyte-agglutinating ability of this tissue glycoprotein. Proteins exhibiting such agglutinating activity, collectively known as lectins, have been isolated from a variety of plants, invertebrates, and lower vertebrates (*3*). To our knowledge the rabbit hepatic binding protein (HBP) described herein is the first lectin of mammalian origin.

The protein was isolated from rabbit liver and purified by affinity chromatography on a Sepharose-asialoorosomucoid column (*4*). It was eluted by a solution of 1.25*M* NaCl, 20 m*M* ammonium acetate, and 0.5 percent Triton X-100, at *p*H 6.4, and extensively dialyzed against 20 m*M* ammonium acetate and 0.25 percent Triton X-100, at *p*H 6.4. To the dialyzed solution cadmium acetate was added to a final concentration of 20 m*M*. The solution was then kept for 30 minutes at 0°C; the precipitated protein was separated by centrifugation for 15 minutes at 10,000*g*, suspended in 2 m*M* cadmium acetate, recentrifuged, and resuspended in a solution of 1.25*M* NaCl containing 20 m*M* ammonium acetate, at *p*H 6.4. Solubilization, effected by the addition of one part of 0.2*M* disodium EDTA to 100 parts of suspension, was followed by a final ammonium sulfate precipitation of the protein and dialysis (*4*). The HBP, prepared in this way, is polydisperse, as characterized by electrophoresis, ultracentrifugation, and chromatography (*4*). The criteria by which purity of this polydisperse protein is estimated are complex (*4*).

Agglutination of red cells by HBP requires the presence of intact sialic acid residues on HBP, a *p*H above 6.5, and at least 0.003*M* calcium ions—conditions also essential to the ability of HBP to bind desialylated glycoproteins in vitro (*4*). Both the agglutination and

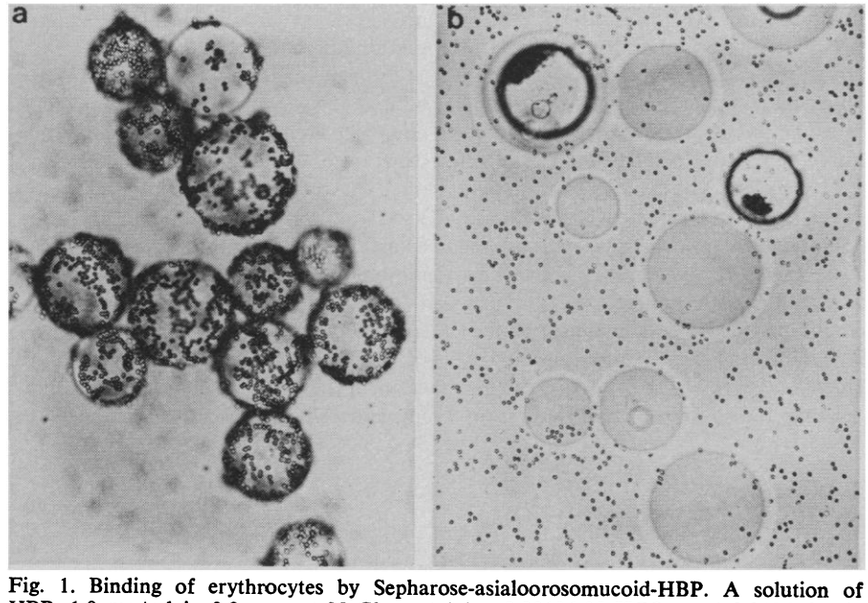

Fig. 1. Binding of erythrocytes by Sepharose-asialoorosomucoid-HBP. A solution of HBP, 1.0 mg/ml in 0.9 percent NaCl, containing 0.001*M* $NaHCO_3$, and 0.01*M* $CaCl_2$, *p*H 7.5, was passed through a small column containing 1.0 ml of CNBr-activated Sepharose 4B to which 3.0 mg of human asialoorosomucoid had been attached (*4*). After the column was washed with the same solution, the suspension of Sepharose beads was incubated for 10 minutes with either (a) human group A or (b) mouse erythrocytes (× 150).

Table 1. Specificities of agglutinating activity of HBP. Erythrocytes from rats, rabbits, mice, and guinea pigs were prepared from heparinized blood, and those from humans were obtained from outdated citrated human blood. The cells were washed three times with a 0.15*M* NaCl solution containing 1 m*M* $NaHCO_3$, and suspended in the same solution made 10 m*M* in $CaCl_2$. To 0.2 ml of a 2 percent suspension of erythrocytes in this solution, 0.05 ml of 1.0 m*M* tris·HCl buffer, *p*H 7.5, containing varying amounts of HBP, were added. The suspension was incubated for 10 minutes at 22°C, and the presence or absence of agglutination was determined microscopically. Agglutinating activity was defined as the reciprocal of the lowest concentration (μg/ml) of HBP resulting in agglutination of at least 10 percent of the cells; complete agglutination was usually effected by twice that concentration. The same degree of agglutination was effected with rabbit erythrocytes by concanavalin A (Sigma); (10 μg/ml; agglutinating activity, 0.10) substituted for HBP. Inhibitory activity, defined as the reciprocal of the lowest monosaccharide concentration (μmole/ml) inhibiting agglutination completely, was assayed by adding varying amounts of monosaccharides to the solution 15 minutes prior to the addition of erythrocytes.

Erythrocytes	Agglutinating activity of HBP			Inhibitory activity of monosaccharides		
	Native	Trypsinized*	Neuraminidase*	*N*-Acetylgalactosamine	D-Galactose	L-Fucose
Human, A	1.25	8.33		2	0.2	0.050
B	0.50	5.00		4	0.3	0.025
H(O)	0.25	3.30		8	0.4	0.013
Rabbit	1.25	8.33				
Rat	< 0.02	< 0.02	1.0			
Mouse	< 0.02	< 0.02	1.0			
Guinea pig	< 0.02	< 0.02	1.0			

* Washed packed cells were suspended in an equal volume of 0.05*M* phosphate buffer, *p*H 7.5, containing either (i) bovine trypsin (Boehringer) (0.25 mg/ml) or *Clostridium perfringens* neuraminidase [5 μg/ml; 1.5 unit/mg (Worthington)]. Incubation, at 37°C, lasted 1 hour with trypsin and 15 minutes with neuraminidase; after incubation the cells were washed with 0.9 percent NaCl 0.001*M* $NaHCO_3$ solution.

binding are reversibly abolished either by reducing the *p*H below 6.0 or by chelating the calcium ions with EDTA. Exposure of HBP to neuraminidase abolishes its asialoglycoprotein binding activity (*4*), and also abolishes its red cell agglutinating activity (*5*).

Table 1 shows that HBP agglutinates native human and rabbit red cells. Quantitative differences between the three human blood groups suggest that interaction of HBP with these cells is through blood group antigens. The HBP agglutinates group A erythrocytes, of which the antigenic carbohydrate terminates in an α-linked *N*-acetylgalactosamine (*6*), more actively than group B or H(O) cells, whose antigens terminate respectively in D-galactose or L-fucose. Agglutination may be reversed by the addition of any of these monosaccharides, but the addition of *N*-acetylglucosamine, D-glucose or D-mannose (all at 50 μmole/ml) was without inhibitory effect.

Table 1 also shows that HBP is incapable of agglutinating untreated rat, guinea pig, or mouse erythrocytes. However, HBP can agglutinate these cells after they have been treated with neuraminidase. Similarly, HBP is unable to bind plasma glycoproteins unless their terminal sialic acid residues have been removed with neuraminidase. Although mild exposure to trypsin significantly increases the agglutinability of human and rabbit erythrocytes, it has no effect on untreated mouse, guinea pig, or rat cells.

Prior incubation of 6.4 μg of HBP with 0.425 μg of asialoorosomucoid—equivalent to 112 percent of the binding capacity of HBP—resulted in the complete loss of erythroagglutinating activity. If 5.0 μg of orosomucoid or asialotransferrin, neither of which produces inhibition of asialoglycoprotein binding (*4*), were substituted for the asialoorosomucoid, erythroagglutinating activity of HBP was unimpaired. These findings confirm the identity of the protein binding and cell agglutinating sites of HBP.

Agglutinating activity requires a minimum of two binding sites located on either a single molecule of HBP or on a molecular aggregate of this protein. When HBP is bound to Sepharose-asialoorosomucoid gel, not all of its binding sites are involved in the complex since about a third of its asialoprotein binding capacity remains. Furthermore, beads of this gel complex are capable of binding human or rabbit erythrocytes but not untreated mouse, guinea pig, or rat erythrocytes (Fig. 1).

The fact that HBP can specifically bind certain sugar moieties of glycoproteins, whether present in solution or as constituents of cell surfaces, suggests that, in addition to its role in carbohydrate and glycoprotein metabolism, such binding may be a factor in certain cellular interactions as well.

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