Such nuclei seem to be limited to breeding frogs—females with ripe ovaries and calling males taken at the breeding sites. Polyploidy seems to be limited to breeding females in *Hyla crucifer*. The six species that did not show polyploidy were all taken outside the breeding season.

This very high incidence of nuclei with amounts of DNA corresponding to higher polyploid classes, and the possible correlation with the reproductive cycle, should make these species interesting subjects for cytological and physiological studies.

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Homoreactant: A Naturally Occurring Autoantibody in Rabbits

Abstract. *Homoreactant, a factor found in the serum of every normal rabbit tested thus far, reacts specifically with buried antigenic determinants on the Fab-fragment of papain-digested autologous and homologous γG-globulin. It appears that rabbits normally produce naturally occurring autoantibodies.*

The products of enzymatic digestions and controlled reductions and alkylations have proved exceptionally useful for describing the chemical and biological nature of the γG-globulin molecule. Of particular importance was the finding that papain digestion of rabbit antibody results in the formation of two antigenically and biologically distinct fragments, Fab and Fc. The crystallizable Fc-fragment has most of the biological properties of the intact antibody molecule; but the Fab-fragment has the antibody-combining site and, although univalent, retains its capacity to react with specific antigens (*1*).

Erythrocytes which are sensitized with immune Fab-fragments resemble erythrocytes sensitized with the incomplete antibodies to Rh (+) erythrocytes in that they do not agglutinate in saline, but they can be agglutinated by a suitable antibody to γG-globulin (*2*). Subsequently we observed that human O Rh (+) erythrocytes sensitized with Fab-fragment from immune rabbits could be agglutinated by a rabbit antiserum to human γG-globulin. Initially we believed that this was due to a cross-reacting antigenic determinant common to human and rabbit γG-globulin. However, we later found this peculiar antiglobulin factor in pooled or individual normal rabbit serum and attributed the activity to a heat-stable, nondialyzable, mercaptoethanol-resistant, γG-globulin factor (*3*). Because this factor differs from other antibodies (such as those called "anti-antibody," antiglobulin reagent, rheumatoid factor-like antibody, and antiallotype antibody), we initially assigned to it the term "homoreactant" to designate a normal γG-globulin that reacts with a fragment (Fab) derived by enzyme digestions of the γG-globulin of the same species (*3*).

We now report results obtained when the homoreactant of individual rabbit serum was tested against the Fab-fragments of γG-globulin isolated from the same rabbit. Our results in Tables 1 and 2 show, by agglutination and inhibition of agglutination, that these individual serums react with autologous as well as homologous Fab-fragments. From these findings we conclude that homoreactant has certain characteristics of an autoantibody whose specificity is directed toward buried autoantigenic determinants unique to the Fab-fragment.

Rabbit antibody to human erythrocyte (HEA) was produced in several rabbits by intravenous injections of washed, human O Rh (+) erythrocytes. The Fab-fragments of antibody to HEA were obtained after papain digestion of γG-globulins isolated from a pool of bleedings from several immune rabbits or a pool of bleedings from each of three individual immune rabbits (A9, A11, and A12). Serums were also collected from these individual rabbits prior to immunization. For the inhibition experiment the Fab-fragments were obtained from papain digests of γG-globulin isolated from several individual bleedings of six normal rabbits (B4, H2, H3, G3, G4, and G5). Precipitations with sodium sulfate and diethylaminoethyl cellulose (DEAE) chromatography were used to purify the γG-globulin from normal and immune rabbit serums (*4*). Papain digestions were performed according to the method of Porter (*5*), and the Fab-fragments were purified as described by Mandy, Stambaugh, and Nisonoff (*6*). No agglutination occurred when dilutions of Fab-fragments from antibody to HEA were mixed with O Rh (+) erythrocytes in saline. Sensitization of the erythrocytes by the Fab-fragments, however, was demonstrated by agglutination reactions with goat antibody prepared against rabbit γG-globulin.

Table 1. Agglutination by normal serums of erythrocytes sensitized with the homologous and autologous Fab-fragment of antibody to HEA. All serums were heat-inactivated and absorbed for heteroagglutinins.

Serums before immunization	Reciprocal dilutions of serums				
	16	64	256	1024	Saline
A9, Fab from antibody to HEA					
A9	4+	2+	1+	0	0
A11	3+	1+	0	0	0
A12	4+	3+	1+	0	0
A11, Fab from antibody to HEA					
A9	4+	3+	1+	0	0
A11	3+	1+	1+	0	0
A12	4+	3+	1+	0	0
A12, Fab from antibody to HEA					
A9	4+	3+	1+	0	0
A11	4+	1+	1+	0	0
A12	4+	4+	2+	0	0

Equal volumes of a 2-percent suspension of washed human O Rh (+) erythrocytes and solutions of Fab (0.31 mg/ml) from antibody to HEA were mixed and incubated at 37°C for 1 hour. The sensitized cells were then washed three times with saline and reconstituted in saline to a 2-percent suspension.

Agglutination reactions were performed by mixing equal volumes (0.1 ml) of each of a serial fourfold dilution of serum and of sensitized erythrocytes. After incubation at room temperature for 15 minutes, the mixtures were centrifuged in a Serofuge (Clay-Adams), and each was scored for agglutination between 0 and 4+.

Inhibition of agglutination was carried out by incubation of equal volumes of various dilutions of the inhibitor with a standard 1:8 dilution of normal serum for 1 hour at 37°C. Sensitized cells were then added, in-

Table 2. Inhibition of serum-agglutinating activity by homologous and autologous Fab. Dilutions of inhibitor were prepared from stock solutions containing 10 mg of protein per milliliter. The saline controls indicate the degree of agglutination by the rabbit serums in the absence of inhibitor.

Fab inhibitor	Inhibitor concentration (reciprocal dilution)				
	2	8	32	128	Saline
	G3 rabbit serum				
G3	0	1+	2+	3+	3+
G4	0	2+	3+	3+	
G5	0	1+	3+	3+	
	G4 rabbit serum				
G3	0	1+	3+	4+	4+
G4	0	1+	2+	4+	
G5	0	1+	3+	4+	
	G5 rabbit serum				
G3	0	tr	1+	2+	2+
G4	0	tr	1+	2+	
G5	0	0	1+	2+	
	B4 rabbit serum				
B4	0	1+	3+	3+	3+
H2	0	1+	3+	3+	
H3	0	1+	2+	3+	
	H2 rabbit serum				
B4	0	0	1+	3+	3+
H2	0	0	1+	3+	
H3	0	0	1+	3+	
	H3 rabbit serum				
B4	0	1+	1+	3+	3+
H2	0	0	2+	3+	
H3	0	0	1+	3+	

cubated for an additional 15 minutes at room temperature, and scored for agglutination.

The results (Table 1) show that before immunization the serums of individual rabbits agglutinate erythrocytes which are sensitized with the Fab-fragment from antibody to HEA. The agglutinating titers are about the same for all the rabbits and for all the Fab sensitizers. It is important to note that each of these serums reacted with erythrocytes sensitized with autologous Fab. Since these serums have been inactivated by heat and absorbed with unsensitized cells, the activity cannot be attributed to immunoconglutinins (*7*) or heteroagglutinins. No agglutination occurred when these serums were tested against rabbit erythrocytes sensitized with rabbit incomplete isoantibody (*8*). Thus, the activity cannot be due to "anti-antibody" (*9*) or to the "agglutinator" described by Cohen and Tissot (*10*). We feel that the factor responsible for the agglutinating activity, recorded in Table 1, is homoreactant because of its occurrence in normal serum, its 7*S* γG-globulin nature, and its specificity for the Fab-fragments.

In contrast to anti-antibody, homoreactant can be inhibited by the Fab of normal γG-globulin (*3*). It appears from previous studies that the reactive sites on the homoreactant are occupied by the Fab and are thus unavailable for reaction with the Fab used to sensitize the erythrocytes (*11*). In Table 2, the saline controls indicate the degree of agglutination obtained by a 1:8 dilution of normal serum in the absence of the inhibitor. Although each serum is inhibited by Fab from various sources, there does not appear to be any significant quantitative or qualitative variation in the degree of inhibition. These results demonstrate in another way that homoreactant is capable of reacting with autologous Fab.

These are representative data taken from a study, during which we have shown that rabbits have a naturally occurring antibody, homoreactant, whose specificity is directed to the Fab-fragment of homologous γG-globulin (*11*). Individual serums from more than 100 rabbits have been tested and all had some measurable homoreactant activity. In extending these findings we now show that homoreactant is capable of reacting with autologous Fab.

An increasing body of reported evidence conflicts with the widely accepted concept that a given animal will not produce antibodies to self-constituents, that is, autoantibodies. These investigations have demonstrated autoantibodies in human patients who suffer from a number of so-called "autoimmune" disorders, and these autoantibodies are generally thought to arise as a result of tissue damage and the subsequent release of autoantigenic substances not usually accessible to the circulation or to sites of antibody production (*12*). There are also many reports of experimentally induced autoimmune diseases in laboratory animals that then show characteristic pathological symptoms and also elicit characteristic autoantibodies (*13*).

It has already been demonstrated that autologous γG-globulin is capable of autoantigenicity, provided there is a sufficient degree of denaturation (*14*). Antibodies produced in rabbits against autologous denatured γG-globulin or autologous papain-digested γG-globulin (*14*) react with heterologous as well as homologous γG-globulins. However, these investigations (*14*) were not concerned with the question of whether such antibodies were capable of reacting with autologous native or papain-digested γG-globulin.

Homoreactant differs from the autoantibodies found in clinical or experimental pathologic serums and from the antibodies produced by immunization with autologous denatured or enzyme-cleaved γG-globulin in that the rabbit serums which we have tested were obtained from rabbits which suffered no apparent abnormality and which had never been artificially immunized.

A similar factor in certain human serums was also shown to have specificity for buried determinants which are exposed by treating human γG-globulin with pepsin (*15*). We are finding that normal human serums also have homoreactant-like activity and suggest that these factors are also autoantibodies.

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