action of lymphocyte and target cells in tissue culture. Lymphocytes from already immunized allogeneic donors aggregate around appropriate target cells in vitro and kill them, whereas allogeneic lymphocytes from untreated animals neither aggregate nor kill (*7*). Unsensitized allogeneic lymphocytes are cytotoxic as well, provided that they are aggregated around the target cells, for example, by phytohemagglutinin (*8, 9*) or by a rabbit antiserum to mouse cells (*9*). When a cultured mixture of target cells and already sensitized lymphocytes was treated with cortisone, the lymphocytes still aggregated around the target cells but were no longer cytotoxic (*10*). Specific aggregation in the presence of cortisone indicates that antibody is still present at the surface of the lymphocytes, but the absence of killing suggests that the cytotoxic effect is not due entirely to fixation of such antibody. Possibly the cytotoxic effect of lymphocytes is due to the same mechanism (as yet obscure) that is responsible for the phenomenon termed allogeneic inhibition, and the function of specific antibody present at the surface of the lymphocyte may be to establish close contact with the target cells (*3, 9*). One would then postulate that cortisone either inhibits the ability of allogeneic antigens and target cells to interact or makes the target cells resistant to the cytotoxic effect of such an interaction.

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Nonspecific Binding of Complement by Digestion Fragments from Antiviral Gamma Globulin

Abstract. *The nonspecificity of rabbit γ-globulin (antibody) to western equine encephalitis virus and the nonspecificity of normal rabbit γ-globulin in complement-fixation tests with antigens prepared from chick-embryo cells infected with this virus and normal chick-embryo cells resided primarily in Porter's fragment III. Addition of complement to fragment III from the antibody globulin, followed by inactivation of the added complement, abolished the complement-fixing ability of fragment III with both specific and nonspecific antigens. Similar treatment of the undigested antibody abolished its complement-fixing ability with nonspecific antigen only.*

Taran (*1*) reported that the anticomplementary effects of an antiserum could be abolished by the addition of complement (C′) to the serum at 37°C and by then heating the mixture to 56°C to inactivate the added C′. When added to fresh C′ such treated antiserum no longer showed anticomplementary activity, but when mixed with its specific antigen it bound C′ as would be expected.

We report the results obtained with fragments of normal rabbit γ-globulin and that from rabbits immunized with western equine encephalitis (WEE) virus in the aforesaid system. The preparation of the antiserum pool, isolation of γ-globulin, and the preparation and isolation of papain-digestion fragments have been described (*2*); however, fragment III was further purified by recrystallization two to three times in the cold. The pepsin-digestion fragment was obtained by the method of Nisonoff *et al.* (*3*). The papain digest (in 0.1*M* acetate buffer, *p*H 5.5) on analytical centrifugation showed one peak at 3.3*S* and the pepsin digest (in borate-buffered saline at *p*H 8.0, ionic strength 0.16) showed one main peak at 4.3*S* plus a small amount of slower-moving material (56,100 rev/min, 20°C, 0.7 percent protein). There was no evidence of a faster-moving component in either preparation. All products showed a single line by Ouchterlony analysis when checked with goat antiserum to rabbit γ-globulin; papain-digestion fragments I and II and the pepsin digest all showed identity with each other, whereas fragment III differed from all the others. All products showed a cross-reaction with the undigested γ-globulin. The complement-fixing antigen in the encephalitis virus was prepared from infected chick-embryo tissue culture by a method described for herpes simplex virus (*4*), with the exception that just prior to the C′-fixation test the cell debris from the crude tissue-culture lysate was removed by centrifugation. Thus, the virus antigen was essentially a crude extract of infected chick-embryo cells plus the culture medium (2 percent lamb serum, 0.5 percent lactalbumin hydrolyzate, 97.5 percent Eagle's basal medium prepard in Hanks balanced salt solution). The antigen used for specificity control was prepared from normal chick-embryo tissue culture in the same manner as for the virus antigen. The complement-fixation tests were performed by a modification (*5*) of the Takatsy (*6*) microtitration, with Kolmer's saline as diluent. The results were recorded as 0, 1+, 2+, 3+, and 4+, according to the degree of inhibition of hemolysis in the indicator system. End points, based on presence (size) or absence of a "button" of red blood cells plus the degree of hemolysis in the supernatant fluid, were read with the naked eye. Results in Tables 1 and 2 are expressed as reciprocals of the highest initial dilution giving at least a 2+ reaction, corresponding roughly to 50-percent hemolysis as end point. All protein samples were heat-inactivated at 60°C for 30 minutes prior to testing.

Samples (0.15 ml) of those preparations showing complement fixation were mixed with the rehydrated solution of lyophilized guinea pig serum (0.05 ml) and incubated at 37°C for 30 minutes. This mixture, after a fourfold (0.6-ml) dilution with Kolmer's saline, was incubated at 60°C for 30 minutes to inactivate the guinea pig complement. The complement-fixation test was then repeated with these treated samples, with active guinea pig serum and virus antigen or normal control antigen (Tables 1 and 2). The same results were obtained when the procedure was reversed—for example, when complement was heated to 60°C for 30 minutes, then added to the sample, and further incubated at 37°C for 30 minutes.

The fragments I and II of antibody γ-globulin to the virus, which contain one antibody-combining site (*7*), and the pepsin-digestion fragment, which

Table 1. Fixation of complement by digest fragments (Fr) of antibody γ-globulin to western equine encephalitis (WEE) and of normal γ-globulin.

Digestion	Protein (%)	C'f titer		
		Antigen*		AC Control†
		WEE	Normal	
γ-Globulin (antibody) to virus				
Papain				
Fr I	0.2	0‡	0	0
Fr II	0.2	0	0	0
Fr III	0.2	256	256	16
None (control)§	0.2	128	128	32
Pepsin	0.7	0	0	0
None (control)§	0.6	≧512	≧512	128
γ-Globulin from serum of normal rabbit				
Papain				
Fr 1	0.2	0	0	0
Fr II	0.2	0	0	0
Fr III	0.1	64	64	8
None (control)§	0.2	128	128	32
Pepsin	0.6	0	0	0
None (control)§	0.5	≧512	256	64

* Antigen prepared from WEE-infected chick-embryo cells and normal chick cells. † Anticomplementary control on each sample. ‡ No C′ fixation (C′f) observed at 1:4, the initial dilution. None of these specimens caused hemolysis in the indicator system. Dilution made in serial twofold steps. § γ-Globulin carried through identical steps as digestion mixtures, with the exception of the addition of enzymes.

contains two antibody-combining sites (*3*), did not fix complement in any of the tests. From the same γ-globulin, fragment III, which contains no antibody-combining site but which has a binding affinity for C′ (*8*) and tissue (*9*), was anticomplementary (that is, there was inhibition of hemolysis in the indicator system by the sample alone) in some tests; in these, the anticomplementary titers varied between preparations from 1 : 4 to 1 : 16. We obtained similar results with normal γ-globulin. In addition, fragment III from both the antibody γ-globulin and normal γ-globulin bound more C′ in the presence of virus antigen or normal antigen (that is, tissue-culture components) than in their absence (anticomplementary titer). This result was constant, the titers in different tests varying from 128 to 256. Possibly, because of the affinity of fragment III for tissue-culture components, aggregates with C′-binding ability were formed. Ishizaka (*10*) reported that aggregation of fragment III by coupling with diazotized benzidine (bis) resulted in complement fixation by the resulting aggregates. Undigested antibody γ-globulin and normal γ-globulin (Table 1) were also anticomplementary and bound C′ nonspecifically. The anticomplementary titers varied in different tests as did the anticomplementary titers of fragment III; for example, if the anticomplementary titer of the undigested sample was low, that of fragment III was also low.

Table 2. Specificity of C′ fixation by digest fragments of antibody γ-globulin to WEE virus and of normal globulin after treatment with guinea pig serum. The sample was treated as follows: Prior to C′-fixation (C′f) test, a portion was reacted with undiluted active guinea pig serum at 37°C for 30 minutes, then diluted to final fourfold dilution with Kolmer's saline and incubated at 60°C for 30 minutes.

Treated sample	Protein %	C'f titer		
		Antigen*		AC Control†
		WEE	Normal	
γ-Globulin antibody to WEE				
Fr III	0.2	0‡	0	0
Papain	.2	64	0	0
Pepsin	.6	256	4	0
γ-Globulin from normal rabbit serum				
Fr III	0.1	0	0	0
Papain	.2	0	0	0
Pepsin	.5	4	4	0

* † ‡ § as listed in Table 1.

Upon prior treatment of the sample with guinea pig serum (Table 2) all significant complement fixation disappeared except for the specific reaction between the undigested antibody γ-globulin to the virus and the virus antigen. Possibly all sites on fragment III capable of binding C′ in either the presence or absence of tissue were now saturated with inactivated complement. Inasmuch as the protein concentration of fragment III from γ-globulin to virus and that of the undigested γ-globulin were comparable (0.2 percent), the amount of fragment III in the former was approximately three times as much as in the latter. Hence, if all of the C′-fixing sites on fragment III isolated from the antiserum to virus were saturated with inactivated C′ then all the C′-reactive sites on fragment III still attached to the undigested molecule were also saturated. Only those C′-combining sites newly created as a result of specific antigen-antibody combination were available for complement binding. However, even though fragment III may have been saturated with inactivated C′, its attachment to the antibody molecule still appeared to be necessary for specific C′ binding, since the pepsin digest, lacking fragment III, did not fix complement (Table 1). The spatial relations of the peptide chains of the pepsin-digestion fragment after attachment to the specific antigen may be quite different from that of the whole antibody molecule, where the presence of fragment III may exercise an orienting influence in the antigen-antibody complex. Thus, the function of fragment III in the intact molecule may be not its C′-binding properties but rather an orienting influence on the configuration of the antigen-antibody complex. Ishizaka (*10*) has suggested that, when antibody and antigen combine, changes in the molecular configuration of fragment III may occur. In such a case, new sites for C′ binding might become available which in the papain-digestion fragment III were not available. Another possible explanation for these findings is one expressed by Schur and Becker (*11*), who pointed out that it is not certain whether fragment III contains all or only a part of the complement-fixing site of the whole molecule.

We agree with others (*8*) that the pepsin-digestion fragment (*5S*) usually does not fix C′ when added in solution to specific antigen and C′. However, Schur and Becker (*11*) found that washed specific precipitates of *5S* antibody and antigen fixed 40 percent of the added C′.

In our study, although fragment III seems to be responsible for nonspecific complement binding in a virus-antiserum system in which antigen prepared from tissues is used, its role in specific complement binding in such a system is not clear.

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