## Neutralization of Sensitized Virus by the Fourth Component of Complement

Abstract. *Herpes simplex virus which had been sensitized with IgM antibody was not neutralized by the addition of the purified activated first component of complement. In the presence of an optimum concentration of the first component of complement, however, the sensitized virus was neutralized by the addition of a high concentration of the purified fourth component of complement. Under these conditions, the addition of the purified second and third components of complement failed to enhance virus neutralization. With low concentrations of the fourth component of complement, the addition of the second and third components enhanced virus neutralization.*

Interaction of virus with antibody to virus results in the formation of an infectious virus-antibody complex (sensitized virus) (*1*). This complex can be neutralized by the addition of antibody to γ-globulin (*1, 2*), whole guinea pig complement (*3–5*), or the four classical components of complement (*3*). Studies on the mechanism of neutralization of sensitized virus by antibody to γ-globulin suggested that the interaction of antibody to γ-globulin with antiviral γ-globulin that is attached to the virion resulted in neutralization by more extensive coverage of the surface of the virion than occurred with the antiviral γ-globulin alone (*1, 2, 6*). This pointed to the possibility that the mechanism of neutralization of sensitized virus by complement might be due to increased coverage of the surface of the virion by the attachment of the early components of complement (C1, C4, C2, C3). The present investigation was undertaken to test this hypothesis.

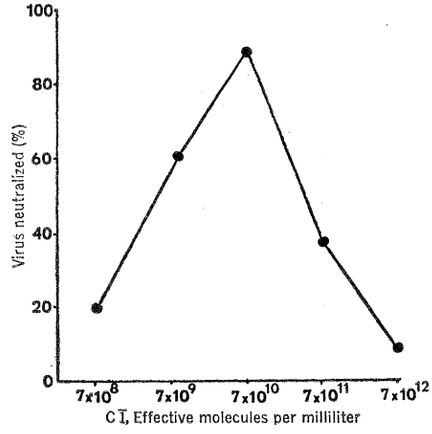


Fig. 1. Neutralization of HSV-IgM by C4 in the presence of different concentrations of C1̄. Portions (0.1 ml) of HSV-IgM (approximately $10^{2.4}$ PFU) were incubated at 30°C with equal volumes of different concentrations of C1̄. At the end of 40 minutes, 0.1 ml of C4 ($3.0 \times 10^{11}$ effective molecules per milliliter) was added to each reaction mixture and incubated for an additional 30 minutes. The reaction mixtures then were assayed for surviving virus, and the percentage of neutralization was determined.

Herpes simplex virus (HSV), strain CHR-HSV-3, was grown in primary rabbit kidney cells and was assayed by its ability to form plaques on these cells (*4*). Early rabbit IgM antibody to HSV was prepared as described (*4*). Serum obtained 9 days after immunization was used in these experiments. The methods of Nelson *et al.* (*7*) were used to prepare functionally pure guinea pig complement (C) components (*8, 9*).

Approximately $10^{7.0}$ plaque-forming units (PFU) of virus were sensitized (*4, 10*) by incubation with undiluted IgM antibody to HSV for 60 minutes at 37°C. The reaction mixture was centrifuged at 41,000*g* for 30 minutes at 5°C. The supernatant was discarded (*11*), and the virus pellet was resuspended in glucose-gelatin-barbital buffer containing $Ca^{++}$ and $Mg^{++}$ (*7*). All dilutions were made in this buffer. Portions of the IgM-sensitized HSV (HSV-IgM) mixture were stored at −70°C.

Initial experiments showed that neither the purified components nor whole guinea pig complement neutralized unsensitized virus. Furthermore, neither activated C1 (C1̄) alone nor C4 alone neutralized HSV-IgM. However, C4 was capable of neutralizing HSV-IgM that had been exposed to C1̄. Maximum neutralization was observed with about $7 \times 10^{10}$ effective molecules of C1̄ (Fig. 1).

In Fig. 2 the percentage of neutralization of HSV-IgM is plotted as a function of C4 concentration in the presence of an optimum amount of C1̄. The data show that, with increased C4 concentration, there was an increase in the amount of virus neutralized. The addition of more than $1.5 \times 10^{11}$ effective molecules of C4 resulted in no significant increase in neutralization. Furthermore, at these high concentrations of C4, the addition of C2 and C3 did not augment neutralization. On the other hand, at low concentrations of C4, the addition of C2 and C3 did augment neutralization. Controls showed that in the absence of either C1̄ or C4, HSV-IgM was not neutralized by C2 and C3 (*12*).

The ability of C4 to neutralize HSV-IgM in the presence of C1̄ represents a hitherto unreported function for the fourth component of C. This observation and the demonstration that, at low concentrations of C4, the addition of C2 and C3 was capable of augmenting virus neutralization support the hypothesis that C neutralizes sensitized virus by the piling up of components on the surface of the virion. The fact that C1̄ alone failed to neutralize HSV-IgM might be due to an inadequate number of C1-fixing sites on the sensitized virion or to the dissociability of activated C1 from these sites. From studies on the action of complement on sensitized erythrocytes, it is known that for each bound molecule of C1̄ more than one molecule of C4 can bind firmly to the cell surface (*13*). A similar amplification step might exist with sensitized virus. This would increase the total number of C molecules which attach to the virion and thereby produce neutralization. An optimum concentration of activated C1 (C1̄), however, is required for effective neutralization of HSV-IgM by C4. The reduced neutralization of HSV-IgM by C4 in the presence of excess activated C1 (Fig. 1) might be ex-

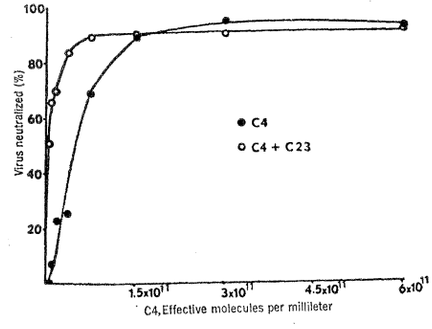


Fig. 2. Effect of different concentrations of C4 on the neutralization of HSV-IgM in the presence and absence of C2 and C3. Portions (0.5 ml) of HSV-IgM (approximately $10^{5.0}$ PFU) were incubated at 30°C with equal volumes of C1̄ ($1.4 \times 10^{11}$ effective molecules per milliliter). At the end of 40 minutes, 0.2-ml portions were removed and incubated for an additional 30 minutes with 0.2 ml of serial twofold dilutions of C4. Each reaction mixture then was diluted with 3.6 ml of glucose-gelatin-barbital buffer, and portions (0.2 ml) were removed and incubated for 30 minutes with equal volumes of the buffer containing C2 and C3 ($3.0 \times 10^{11}$ effective molecules of each per milliliter) or buffer. The reaction mixtures then were assayed for surviving virus and the percentage of neutralization was determined.

plained by the fact that activated C1 can destroy C4 in the fluid phase (*14*), thereby decreasing the amount of C4 that can bind to the virus. In vivo, fluid phase destruction of C4 normally does not occur because C1 exists in the precursor nonactivated state. Thus, in vivo, IgM-sensitized virus should be effectively neutralized by C4.

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8. The complement components were produced under PHS contract PH 43-66-936 by the Cordis Laboratories (Miami, Florida). The nomenclature of complement conforms to that recommended in *World Health Organ. Bull.* **39**, 935 (1968). The purified preparations of $C\bar{1}$, C4, C2, and C3 contained $7.0 \times 10^{12}$, $6.0 \times 10^{11}$, $6.0 \times 10^{11}$, and $3.0 \times 10^{11}$ hemolytically effective molecules per milliliter, respectively (*9*). At the concentration used in our experiments, as determined by "effective molecular titration" (*9*), $C\bar{1}$ contained no measurable amounts of C4, C2, C3, C5, C6, and C7 and less than 1 percent of C8 and C9; C2 contained no measurable amounts of $C\bar{1}$, C4, C3, C5, C6, C8, and C9 and less than 2 percent of C7; C4 contained no measurable amounts of $C\bar{1}$, C2, C3, C5, C6, C7, and C9 and less than 1 percent of C8; C3 contained no measurable amounts of $C\bar{1}$, C4, C5, C7, and C9 and less than 1 percent of C2, C6, and C8.
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11. Initial experiments showed that $C\bar{1}$ was inactivated by the supernatant of the virus-antibody mixture.
12. Tissue culture media, sensitized virus, and primary rabbit kidney cells were assayed (*9*) and found not to contain C2, C3, or C4 activity.
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15. We thank Drs. S. E. Mergenhagen and H. Gewurz for interest and advice.

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## Ventricular Arrhythmias Related to Antibiotic Usage in Dogs

Abstract. *In dogs, pretreatment with the macrolide antibiotic tylosin (5 milligrams per day per kilogram of body weight) increased the incidence of ventricular tachycardia and fibrillation during acute myocardial ischemia. Another group received a dose of acetyl strophanthidin which was nontoxic in controls, but which resulted in a ventricular arrhythmia in six of seven animals on antibiotic treatment. Enhancement of loss of potassium ion from the myocardium by the antibiotic was presumed to be related to the altered cardiac rhythm.*

Despite the widespread use of antibiotics their effects upon the cardiovascular system are undefined. Certain antibiotics are known to influence ion transport in vitro, and may exhibit a selective effect on potassium (*1*, *2*). During in vivo studies the net movement of this ion in the myocardium has been shown to be closely related to the onset of ventricular arrhythmias and restoration of normal sinus rhythm by antiarrhythmic agents (*3*, *4*).

Preliminary observations which suggest a cardiac effect were made in dogs receiving a macrolide antibiotic, tylosin, used in veterinary medicine for control of upper respiratory infection, prior to the induction of myocardial ischemia. The following study was designed to compare animals undergoing ischemia or receiving a digitalis compound, acetyl strophanthidin, either with or without antibiotic.

An electrode catheter was placed in the left anterior descending coronary artery in intact anesthetized dogs as previously described (*4*). All animals were healthy, male mongrels weighing between 19 to 24 kg, and all received morphine sulfate, 3 mg/kg subcutaneously, and Nembutal, 15 mg/kg intravenously. The production of a thrombus that completely occludes the anterior descending artery by this method is associated with an acute, persistent elevation of the ST segment in the pre-

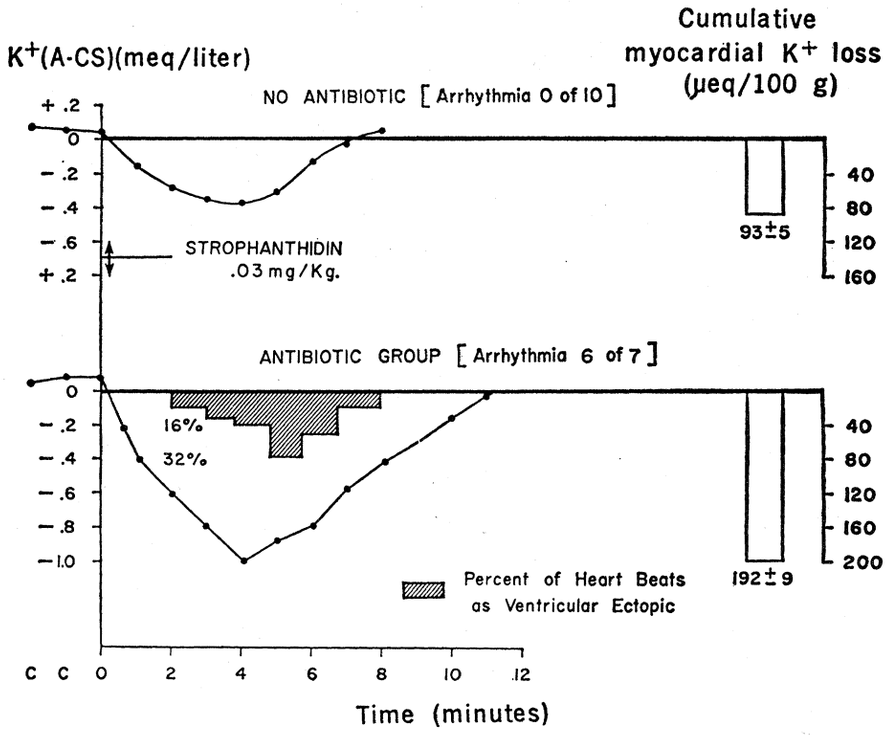


Fig. 1. The upper panel depicts the negative arterial-coronary sinus (*A-CS*) difference of potassium, reflecting myocardial loss of this ion after strophanthidin in the group receiving no antibiotic. The lower panel shows the corresponding changes in the tylosin-treated group, with a larger $K^+$ loss, and a maximum ventricular ectopic beat incidence that averaged 32 percent of all beats. The cumulative ion changes (columns) were calculated from the product of coronary blood flow ($^{85}Kr$ method) and the arterial-coronary sinus difference. *C*, controls at −10 and −5.