STUDIES ON THE ROLE OF COMPLEMENT

IN THE NEUTRALIZATION OF

HERPES SIMPLEX VIRUS

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Summary

Complement was shown to enhance the neutralization of herpes simplex virus. Early and late rabbit anti-herpes sera neutralized virus in the absence of complement, although neutralization was enhanced on the addition of complement. In the literature, these types of antibodies have been classed as non-complement requiring neutralising antibodies (non-CRN antibodies).

Preliminary investigation with early (acute) human anti-herpes serum showed low or negligible neutralization in the absence of complement which was greatly enhanced when complement was added. The late (convalescent) human anti-herpes serum neutralized virus in the absence of complement although again, enhancement occurred when complement was present. Complement-requiring neutralizing antibodies (CRN antibodies) were thus the main types of antibodies present in the early phases after infection with herpes virus and these were later replaced, as the infection progressed, by non-CRN antibodies.

Attempts were made to demonstrate the types and time of appearance of CRN and non-CRN antibodies in mice after immunization with herpes virus. Early mouse anti-herpes serum showed low neutralizing activity in the absence of complement which was only slightly enhanced by the addition of complement. Later sera appeared to contain antibodies of the non-CRN type since neutralization was marked in the absence of complement.

Introduction

It has been known for some time that a factor present in normal serum enhances the action of neutralizing antibodies against various types of viruses. Gordon (1925) working with vaccinia virus showed that heating lowered the neutralizing potency of immune serum. The enhancing effect of a heat-labile substance on the neutralizing activity of immune serum was demonstrated for Rous sarcoma virus (Muller, 1931), Western equine encephalitis virus (Morgan, 1945; Whitman, 1947), mumps virus (Leymaster and Ward, 1949), Newcastle disease virus (Howitt, 1950), dengue fever virus (Sabin, 1950) and variola virus (McCarty and Germer, 1952). Dozois *et al.* (1949) showed that the nature of the heat-labile substance responsible for the enhancing effect on anti-WEE neutralizing antibodies were the second, third and fourth components of complement. The role of complement as the enhancing factor in virus neutralization was firmly established by similar studies with herpes simplex virus (Taniguchi and Yoshino, 1965).

Early and late immune sera differ in their requirement for complement for subsequent neutralization of virus. Thus, studies with early sera obtained from rabbits immunized with herpes simplex virus

showed that these mainly contained CRN antibodies while late sera were non-complement requiring, although complement also enhanced the neutralizing activity of such late antisera (Yoshino and Taniguchi, 1964; Wallis and Melnick, 1971). Similar results were obtained with guinea pig anti-herpes sera (Yoshino and Taniguchi, 1966). Adler et al. (1971) showed that complement amplifies the activity of rabbit anticoliphage early IgM and IgG antibodies up to a thousandfold.

CRN antibodies were also found in the early sera of rabbits artificially infected with herpes virus and in naturally infected man (Yoshino and Taniguchi, 1964; 1966). In cases of herpes virus infections, serum taken early in the disease was shown to neutralize virus only in the presence of complement. This finding has considerable diagnostic value as the presence of CRN antibodies can be taken as evidence of infection. The finding of CRN antibodies in early immune sera thus appears to be a common phenomenon since they have been detected in various species of either immunized or infected animals including man.

This thesis examines the role of complement in the neutralization of herpes simplex virus by early and late antisera raised in rabbits by an immunization scheme which differs from that employed by several authors (Yoshino and Taniguchi, 1964; Hampar et al., 1968; Wallis and Melnick, 1971). The resulting differences obtained, especially with the early sera, will be analysed and discussed. The results obtained with the human acute and convalescent anti-herpes sera were similar to those obtained by Yoshino and Taniguchi (1966).

In order to investigate further the time of appearance of CRN and non-CRN antibodies, mice were immunized with herpes simplex virus and the immune sera obtained at various time intervals were tested in neutralization experiments in the presence and absence of complement. The results were in many respects similar to those obtained by Yoshino and Taniguchi (1964) who used rabbits in their experiments.

Materials and Methods

Cultured cells

BHK21 (baby hamster kidney) cells (Macpherson and Stoker, 1961) were used throughout this study.

Growth medium and diluents

Cells were serially subcultured and maintained in modified Eagle's medium (Vantsis and Wildy, 1962) containing 10% calf serum and 10% tryptose phosphate broth (ETC). Stock virus suspensions were prepared in ETC and, when diluted, the same medium was used as diluent. All sera and complement, whether unheated or inactivated, were diluted in Dulbecco A solution (0.8% NaCl, 0.02% KCl in phosphate buffer, pH 7.0) (Dulbecco and Vogt, 1954).

Virus production

The HFEM strain was used throughout as herpes simplex virus type 1. This strain was also used to immunize rabbits and mice. Herpes simplex virus type 2 (strain 3345) was used in some of the neutralization tests. Both virus types were grown in BHK21 cells. The cells were first allowed to form confluent monolayers in 80 oz. Winchester bottles after which each was infected with 10^7 plaque forming units (p.f.u.) of virus in 20 ml ETC. The virus was allowed to adsorb for 60 min at 32°C and then a further 180 ml of ETC was added and incubation continued for 2 to 3 days. Widespread cytopathic effect was usually observed by this time. After decanting most of the medium, the cells were scraped off into a small volume of remaining ETC and then centrifuged at 1000 rev/min for 10 min. The sedimented cells were resuspended in 5 ml fresh ETC and disrupted with an ultrasonic probe (MSE Ltd., Crawley, Sussex). The disrupted material was then centrifuged at 4000 rev/min for 15 min, the supernatant fluid collected and stored in small volumes at -70°C until used.

Virus assays

Assays were performed by the suspension-plaque method of Russell (1962). Briefly, 2 ml of appropriate virus dilutions were incubated with 7×10^6 BHK cells for 60 min at 37°C on a shaker. Then, 8 ml of 0.8% carboxymethyl cellulose (CMC) in ETC (CMC/ETC), pre-warmed to 37°C, were added and the resulting suspension poured into two 45 mm plastic plates which were subsequently incubated at 37°C in a 5% CO_2-air incubator. At the end of the incubation period (usually 2 days), the medium was decanted and the monolayers washed once with phosphate buffered saline. They were then fixed for 10 min with 10% formol saline. After removal of the fixative, the plates were washed in tap water and then stained with dilute carbol fuchsin for 10 min followed by washing twice in tap water. Plaques were counted with a plate microscope using a $\times 2.5$ magnification.

Neutralization tests

Kinetic neutralization experiments were carried out throughout this study. Unless otherwise stated, the following volumes of reagents and virus titres were used in these tests.

0.05 ml of an appropriate dilution of immune serum was mixed with an equal volume of guinea pig complement diluted to contain 10 units in 0.05 ml volume. 0.1 ml of a suspension of virus containing 5×10^4 p.f.u./ml was then added and the mixture left to incubate at room temperature for the time indicated in the Figures. Then 0.1 ml was diluted in 9.9 ml ETC and residual infectivity was assayed as described under virus assays. Parallel control samples were incubated with pre-immunization serum in corresponding dilutions. The virus-serum mixtures tested in the absence of complement contained inactivated guinea pig serum (i.e., heated at 56°C for 30 min) in corresponding dilutions and volumes and these were also tested in parallel with the samples containing active (unheated) complement.

All sera were heated at 56°C for 30 min before use.

The neutralization rate constant, k, was then calculated from

$$k = \frac{2.303}{ct} \log \frac{v_0}{v}$$

where c is the final concentration of antiserum, t is the time in min of neutralization, v_0 is the initial virus titre and v the residual virus infectivity.

In some Figures, the $\log_{10}$ of the surviving virus fraction is shown plotted against time; in other cases, the residual percentage infectivity (% surviving virus) is shown i.e., the residual infectivity of the virus-antiserum mixture was expressed as a percentage of the infectivity of a mixture with pre-immunization serum at a corresponding dilution and after incubation for the same period.

Anti-herpesvirus sera

a) Rabbit immune sera

These were produced as described by Watson et al. (1966). Briefly, virus was grown in RK13 (rabbit kidney) cells which were then harvested, disrupted with an ultrasonic probe and the suspension finally freeze-dried. The immunizing antigen consisted of 0.2 g of freeze-dried material (equivalent to approximately 6×10^9 p.f.u.) mixed with 1.5 ml of incomplete Freund's adjuvant, the whole made up to 5 ml with sterile water. The resulting emulsion was homogenized and injected intramuscularly. To begin with, seven fortnightly injections were given, the first containing formalinized antigen. Bleedings were made 10 days after each injection and sera separated after standing the blood samples overnight at 4°C. (Sera are given laboratory identification numbers; thus serum 466/I is the sample taken 10 days after the first injection, 466 representing the rabbit number. Serum 466/III is the sample taken from the same rabbit 10 days after the third injection and so on). After the seventh injection, the rabbit was rested for three months and then boosted with 0.1 g antigen. It was then bled after 10 days, this sample representing serum VIII.

For the purpose of discussion in this study, the serum sample obtained from the first bleeding will be referred to as early rabbit serum. Sera taken from the seventh bleeding onwards will be termed late rabbit sera.

b) Human immune sera

Normal pooled human serum and paired sera from a patient (E.P.) were investigated for neutralizing antibodies against herpes virus in the presence of heated and unheated complement. In the latter case, the first blood sample was taken at the time of appearance of the herpetic lesions (early antiserum). The second blood sample was obtained seven weeks after the first and served as the late hyperimmune serum.

c) Mouse immune sera

Fifteen 3-month old mice were divided

Immune sera from rabbit No. 466

Serum No.	HSV type 1			HSV type 2				
	$+c$	$-c$	$\dfrac{k+c}{k-c}$	$+c$	$-c$	$\dfrac{k+c}{k-c}$	$\dfrac{k_1+c}{k_2+c}$	$\dfrac{k_1-c}{k_2-c}$
I	4.8	1.8	2.6	1.4	0.7	2.0	3.4	2.5
III	6.1	2.5	2.4	4.2	1.7	2.4	1.4	1.5
V	8.6	6.3	1.3	5.5	1.7	3.3	1.5	3.7
VII	8.3	3.8	2.1	7.2	3.9	1.8	1.1	1.0
VIII	24.1	20.5	1.1	6.8	3.5	1.9	3.5	5.7

Immune sera from rabbit No. 467

| Serum No.	HSV type 1			HSV type 2				
	$+c$	$-c$	$\dfrac{k+c}{k-c}$	$+c$	$-c$	$\dfrac{k+c}{k-c}$	$\dfrac{k_1+c}{k_2+c}$	$\dfrac{k_1-c}{k_2-c}$
I	6.3	3.8	1.6	1.2	0.3	4.0	5.2	12.6
III	6.6	3.4	1.9	4.9	1.7	2.8	1.4	2.0
V	12.6	6.6	1.9	10.8	6.6	1.6	1.1	1.0
VII	14.7	6.9	2.1	10.8	6.4	1.7	1.3	1.1
VIII	16.6	10.1	1.6	6.6	2.8	2.3	2.5	3.4

Table 1. — The neutralization rate constant (k) values of immune sera from rabbits Nos. 466 and 467 with herpes simplex virus (HSV) types 1 and 2 in the presence ($+c$) and absence ($-c$) of complement.

into three groups and each immunized once with herpes virus type 1, each mouse receiving approximately 10^7 p.f.u. The virus was rendered inactive by treatment with 10% formol saline for 1 h at 37°C and injected intraperitoneally. All the mice in one group were killed and bled on the third day following immunization. The blood was pooled and the serum separated after allowing to stand for some hours at 4°C. The other groups of mice were similarly treated but bleeding was made on the tenth and twentieth day post-immunization.

Complement

Unheated guinea pig serum was used as complement. It was stored at -70°C until used. As indicated previously, heated guinea pig serum at 56°C for 30 min was added to those virus-serum samples which were to be tested in the absence of complement. Titration of complement was performed after the method of Yoshino and Taniguchi (1964). Unheated guinea pig serum was diluted serially in phosphate buffered saline. 0.05 ml of each dilution was then mixed with 0.05 ml of a virus suspension containing the same amount of p.f.u. as that used in the neutralization tests. A sensitized haemolytic system made up of equal parts of 3% washed sheep red cells and rabbit anti-sheep haemolysin containing 2 units was added to all tubes in 0.1 ml amounts. Appropriate controls were also included. After shaking, the tubes were placed in a waterbath
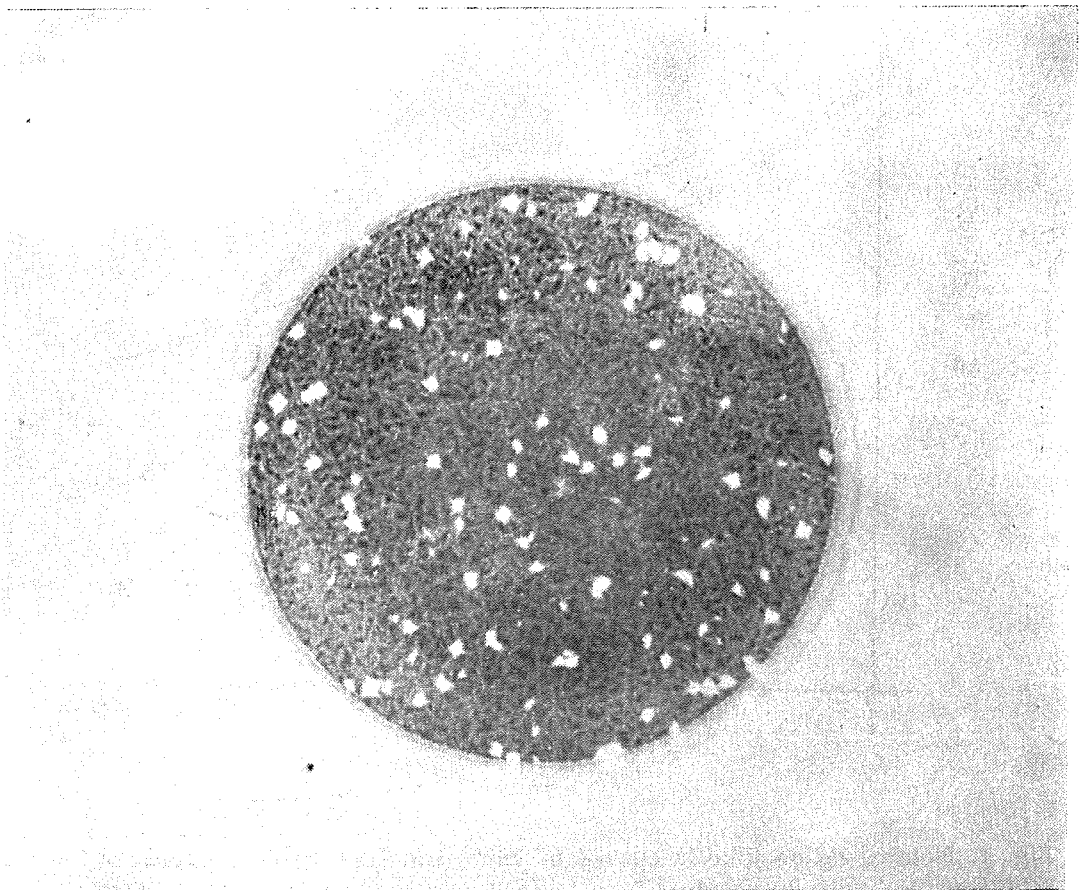
Plaques produced by Herpes simplex virus type 2 on BHK (baby hamster kidney) cells.

at 37°C for 30 min. Reading was made at the end of this period and the highest dilution of guinea pig serum giving complete haemolysis was taken to represent 1 haemolytic unit in 0.05 ml.

Results

Neutralization kinetic tests with rabbit sera

Five successive sera (I, III, V, VII and VIII), each from two rabbits (Nos. 466 and 467), were examined for neutralizing activity against both herpes simplex virus types 1 and 2 in the presence and absence of complement.

Table 1 shows all the data relating to these experiments. It will be seen that the k value of both rabbit sera against type 1 virus both in the presence and ab-

sence of complement was significantly greater than the corresponding value against type 2 virus. This fact can also be seen by examining the last two columns of the Table which relate to the ratio of the k value of type 1 to type 2 virus. This indicates that the sera were more specific to the homologous virus. The $\frac{k+c}{k-c}$ ratio (i.e., the ratio of the k value in the presence of complement to that in the absence of complement) of both sera, against both types of virus, showed no significant difference between the early and later sera. This indicates that no difference existed in complement dependence for neutralization between the early and late sera.

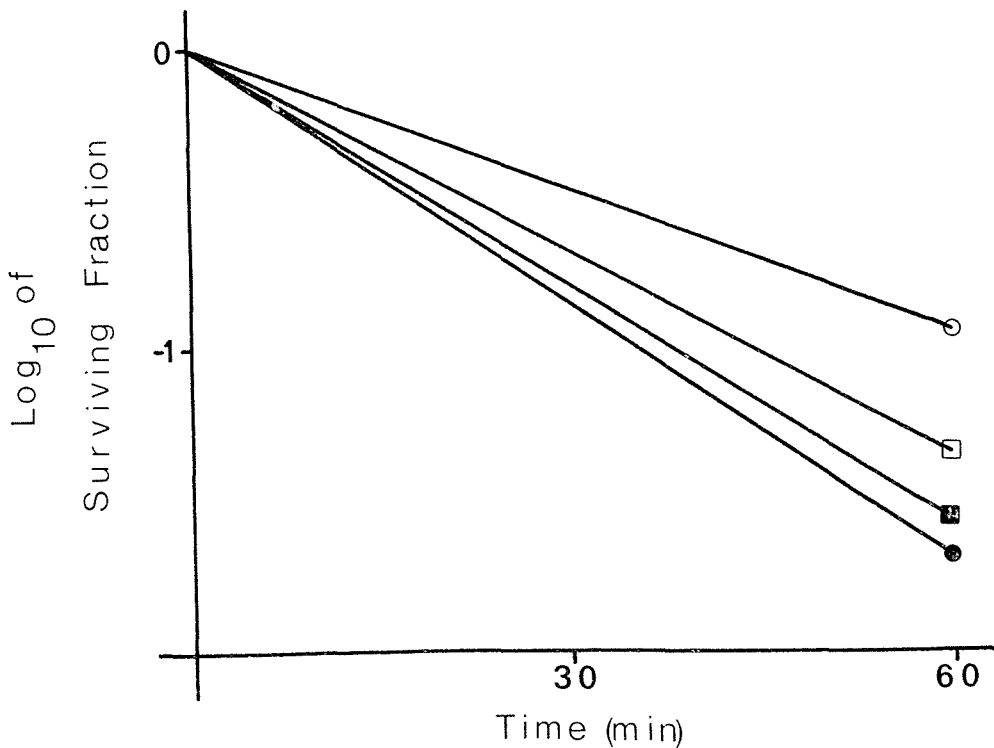Fig. 1 shows the neutralization kinetic curves of early and late sera from rabbit

Fig. 1: Neutralization kinetic curves of early and late serum samples of rabbit no. 466 with herpesvirus type 1. Early serum (diluted 1/10): ○ with inactivated C′ and ● with unheated C′. Late serum (diluted 1/100): □ with inactivated C′ and ■ with unheated C′.

No. 466. Both sera showed a marked neutralizing capacity in the absence of complement which was only slightly enhanced when complement was added. The same effect was also seen when the same sera were tested against type 2 virus and also in tests with early and late sera from rabbit No. 467. Another, perhaps important finding, was that both early rabbit sera showed a relatively high k value when tested in the absence of complement (the average value against type 1 virus was 2.8 and against type 2 virus was 0.5).

The early rabbit sera therefore contained mainly non-CRN antibodies. The late rabbit sera obviously contained similarly non-CRN antibodies since neutralization was marked in the absence of complement. In both cases, neutralization was only slightly enhanced when complement was added.

Neutralization kinetic tests with human sera

Since it is known that a significantly high percentage of normal adults possess anti-herpes antibodies (Burnet and Lush, 1939; Yoshino et al., 1962), pooled human serum was tested for neutralizing activity against herpes virus type 1 both in the presence and in the absence of complement. The result is shown in Fig. 2 It will be seen that the serum had relatively marked neutralizing activity even in the absence of complement.

Since pooled human serum is expected to contain a random mixture of early and late antiherpes antibodies, it was decided to test paired sera from patients known to have had a herpetic infection with a view to find out, if any, differences for complement requirement in neutralization between acute (early) and convalescent (late) sera.
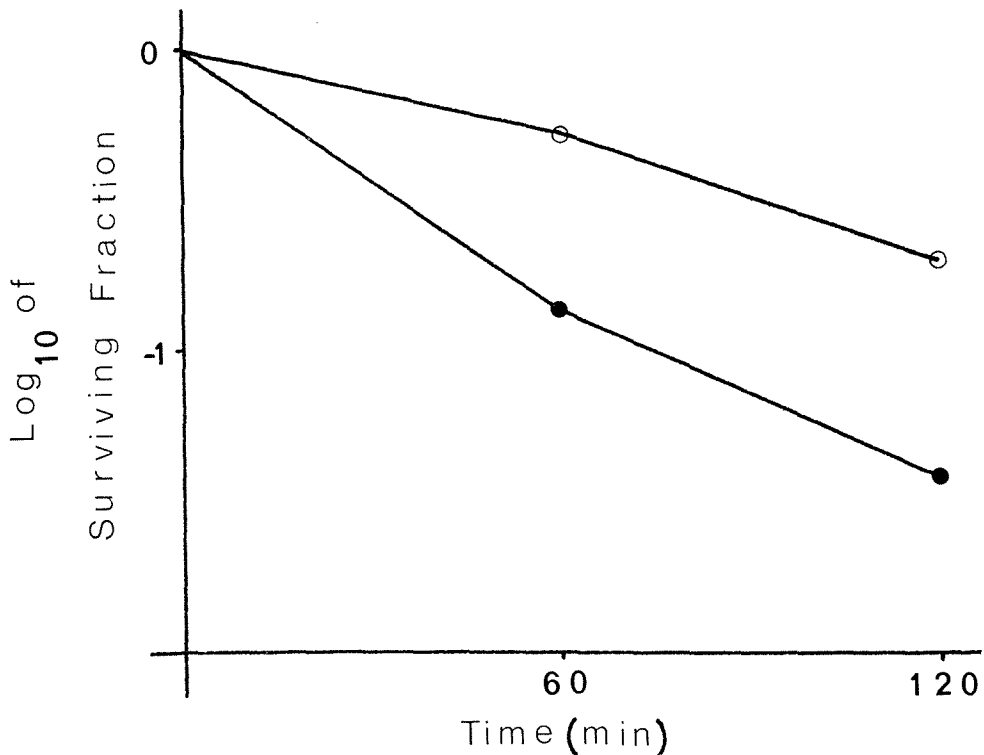

Fig. 2: Neutralization kinetic curves of pooled human serum diluted 1/15: ○ with inactivated C′ and ● with unheated C′.

Human immune sera

Neutralization data with HSV type 2

Serum (type and dilution)	k +c	mean	-c	mean	$\dfrac{k+c}{k-c}$	mean
E 5/5	0.21		0.04		5.25	
E 3/5	0.27	0.34	0.02	0.036	13.50	9.50
E 1/5	0.55		0.05		11.00	
L 5/5	0.56		0.24		2.30	
L 3/5	0.80	0.94	0.35	0.420	2.30	2.24
L 1/5	1.47		0.67		2.20	

Neutralization data with HSV type 1

Serum (type and dilution)	k +c	mean	-c	mean	$\dfrac{k+c}{k-c}$	mean
E 5/5	0.11		0.012		9.10	
E 3/5	0.12	0.17	0.012	0.018	10.00	9.30
E 1/5	0.29		0.030		9.00	
L 5/5	0.45		0.333		1.40	
L 3/5	0.60	0.67	0.311	0.390	1.90	1.70
L 1/5	0.98		0.533		1.80	

Table 2. — The neutralization rate constant (k) values of human early (E) and late (L) sera from patient E.P. with herpesvirus types 1 and 2 in the presence (+c) and absence (−c) of complement.

For early serum: $\dfrac{k_{2+c}}{k_{1+c}} = 2.0$ and $\dfrac{k_{2-c}}{k_{1-c}} = 2.0$

For late serum: $\dfrac{k_{2+c}}{k_{1+c}} = 1.4$ and $\dfrac{k_{2-c}}{k_{1-c}} = 1.2$

Paired sera from a patient (E.P.) were tested. This patient had both genital and oral herpetic lesions and a type 2 virus was isolated. The results of tests for neutralizing antibodies against both virus types and the effect of complement on the neutralization curves are shown in Figs. 3 and 4 and in Table 2. The k values were higher for type 2 than for type 1 virus both in the presence and absence of complement. This finding confirmed that infection was due to a type 2 herpesvirus. The early serum showed only a slight decrease in virus infectivity in the absence of complement. Neutralization was greatly enhanced on the addition of complement. In contrast, the late serum showed a marked neutralizing capacity in the absence of complement which was only slightly enhanced when complement was present. The mean value of the $\dfrac{k+c}{k-c}$ ratio for the early serum was more than 9.0 while that of the late serum was only approximately 2.0.
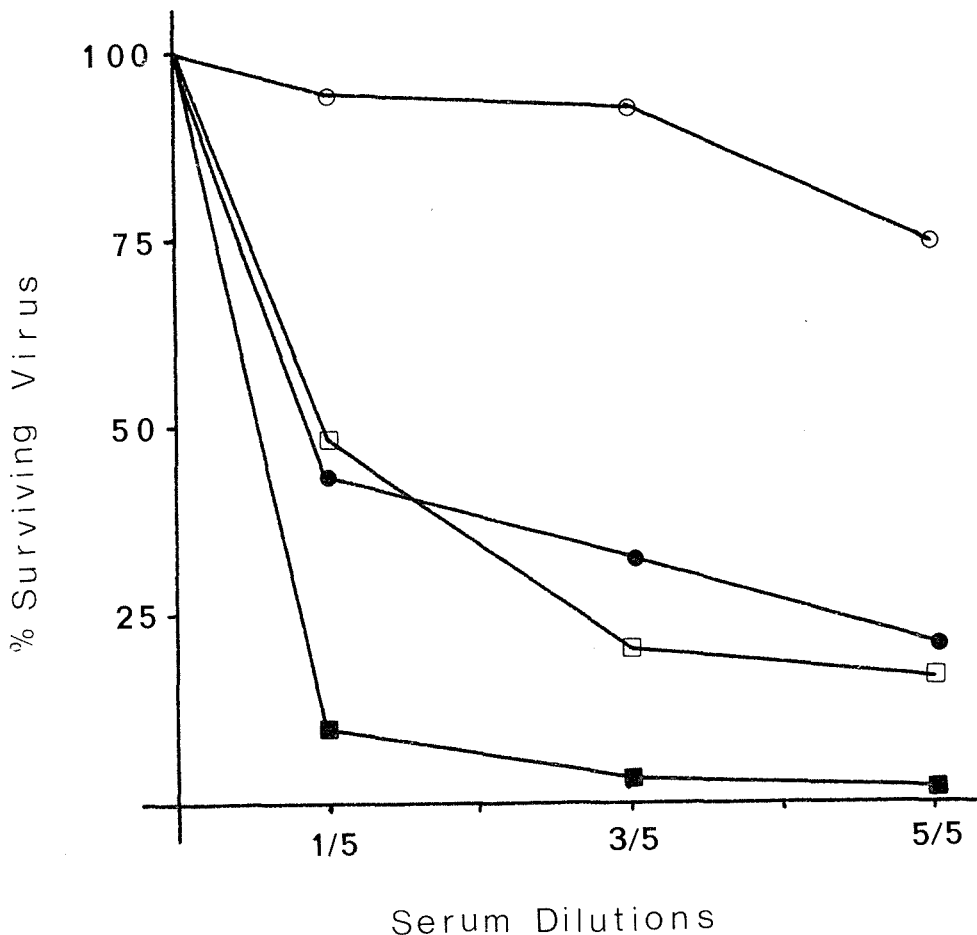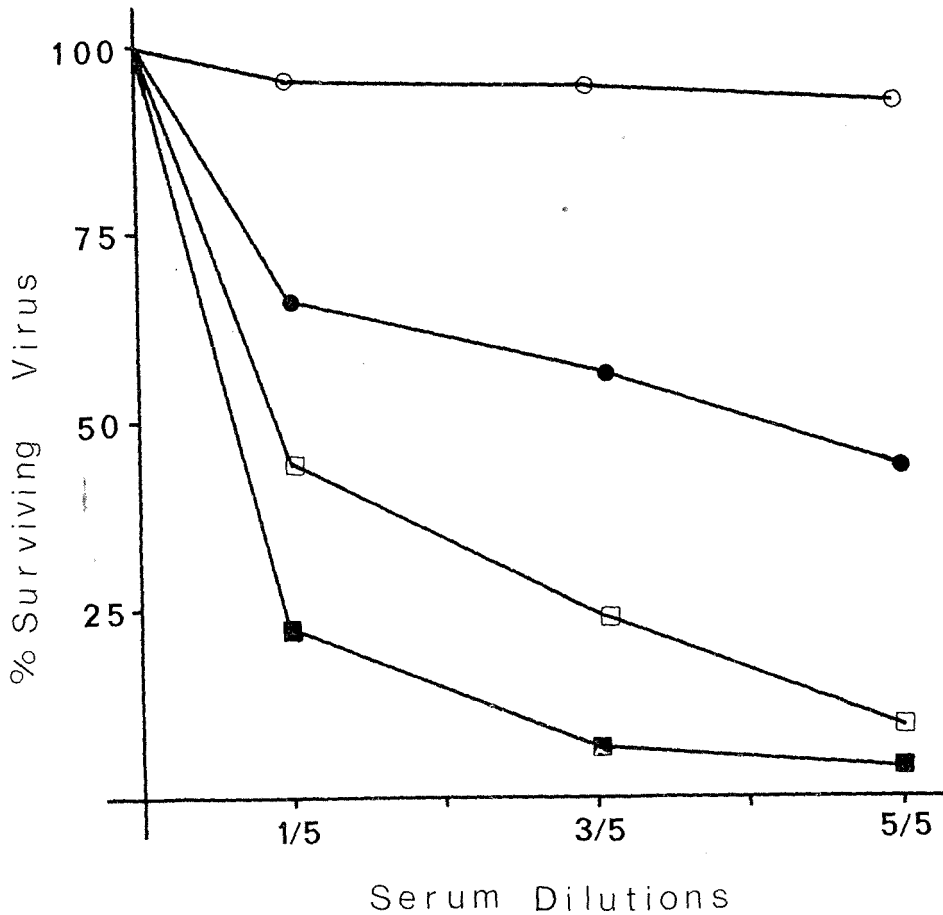

Fig. 3: Neutralization kinetic curves with Herpesvirus type 2 against serum from a patient (E.P.) with a herpetic infection. Surviving virus assayed after 30 min. Early (acute) serum: ○ with inactivated C′ and ● with unheated C′. Late (convalescent) serum: □ with inactivated C′ and ■ with unheated C′.

Fig. 4: Neutralization kinetic curves with Herpesvirus type 1 against serum from a patient (E.P.) with a herpetic infection. Surviving virus assayed after 30 min. Early (acute) serum: ○ with inactivated C′ and ● with un-heated C′. Late (convalescent) serum: □ with inactivated C′ and ■ with unheated C′.

Neutralization kinetic tests with mouse sera

The results with early rabbit and human antiherpes sera differed in that only the latter contained CRN antibodies. The finding that the early rabbit sera did not contain CRN antibodies in contrast to results obtained by several workers (Yoshino and Taniguchi, 1964; Hampar et al., 1968; Wallis and Melnick, 1971) may be due to the different method employed in this study in preparing the immunizing antigen, the route of administration and the time the sample was taken after the immunizing dose.

In an attempt to investigate this point further and to time the appearance of CRN antibodies, if any, mice were immunized with one dose of herpes virus antigen and successive blood samples collected as described earlier. The sera were kept at -20°C until all samples were taken and then tested simultaneously for neutralizing activity in the presence and absence of complement. The results are shown in Fig. 5 and in Table 3. Compared to the early human serum at the same dilution
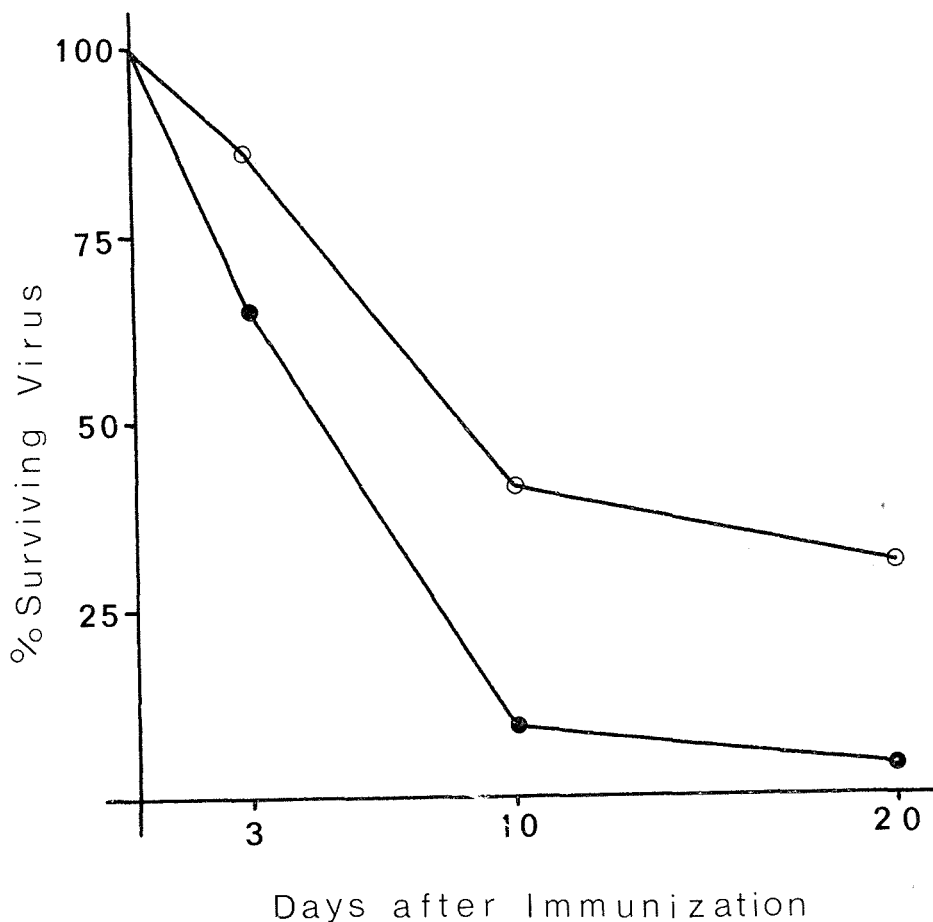
Fig. 5: Neutralization kinetic curves with mouse sera taken 3, 10 and 20 days after immunization with one dose of herpes simplex virus. In each case immune serum was diluted 1/5 and surviving virus assayed after 30 min.

O——O neutralization in the presence of heated (inactivated) C′.

●——● neutralization in the presence of unheated (active) C′.

(Fig. 3), the first mouse serum sample taken on the third day after immunization showed moderate neutralizing activity in the absence of complement which was only slightly enhanced when complement was present. The later serum samples showed a marked neutralizing capacity in the absence of complement and enhancement occurred when complement was added.

The effect of varying concentrations of complement on the neutralizing activity of early rabbit and human sera.

Fig. 6 shows the results obtained in this experiment. Complement was diluted to contain the stated number of units in 0.05 ml. In other respects, the neutralization tests were carried out as described earlier. The early human serum showed little, if any, neutralizing activity in the

absence of complement. Neutralization proceeded on the addition of complement reaching a maximum at the 2.5 unit level after which excess of complement seemed to have no increased enhancing effect. In the case of early rabbit serum, neutralization was marked in the absence of complement and viral infectivity was reduced to approximately 20%. Neutralization was slightly enhanced by complement, again reaching a maximum in the 2.5-5 unit range and then remained constant even in the presence of excess complement.

Discussion

The experiments described in this thesis show that the neutralization of herpes simplex virus is enhanced by the presence of complement.

In most studies cited in the literature, dilution end-point neutralization tests were carried out to differentiate sera containing CRN antibodies from those containing non-CRN ones. In the present study, only kinetic neutralization tests were performed and this, perhaps, makes it more difficult to differentiate these two types of antibodies, especially in 'border-line' cases.

The early rabbit antibodies cannot be classed as complement-requiring for neutralization of herpes virus since neutralization was marked in the absence of complement. These results differ from those obtained by Yoshino and Taniguchi (1964; 1965a), Hampar et al. (1968) and Wallis and Melnick (1971) who showed that early rabbit antiherpes sera contained only CRN antibodies. This observation may reflect differences in the immunization procedures employed. Thus, the immune sera used in neutralization experiments by the above workers were raised by injecting virus intravenously without the addition of adjuvant and the early serum sample collected 7 to 8 days after the immunizing dose. The immune rabbit sera employed in this study were raised by injecting freeze-dried antigen in Freund's adjuvant intramuscularly and the early serum sample collected 10 days after the first immunizing dose. The method employed in preparing the immunizing antigen, the route

Mouse immune sera

Serum	k		$\dfrac{k+c}{k-c}$
	$+c$	$-c$	
3 day	0.29	0.12	2.4
10 day	1.69	0.57	2.9
20 day	2.10	0.78	2.6

Table 3. — The neutralization rate constant (k) values of mouse sera with herpesvirus type 1 in the presence ($+c$) and absence ($-c$) of complement.

Mice were immunized once with herpes virus type 1 and serum samples collected on the 3rd, 10th and 20th day after immunization.

All sera used in this experiment were diluted initially 1/5 and surviving virus assayed after 30 min of neutralization.

of administration and the time of collection of the serum samples may thus be determining factors in the type of antibody which the particular serum may be expected to contain.

In the case of human infections with herpes simplex virus, the results obtained in this study suggest that antibodies present early in infection are dependent on complement for neutralization (CRN antibodies) while late sera contain non-CRN antibodies. These results were similar to those obtained by Yoshino and Taniguchi (1966) who proposed that detection of CRN antibodies in a single sample may become a new method for the early diagnosis of herpetic infections.

The mouse immune sera were raised by injecting antigen intraperitoneally and the earliest serum collected 3 days after immunization. The early mouse antibodies, in the absence of complement, were intermediate in neutralizing potency when compared, under the same conditions, with early human and rabbit antibodies. Enhancement of neutralization by complement was greater with early human than with early mouse serum. Later
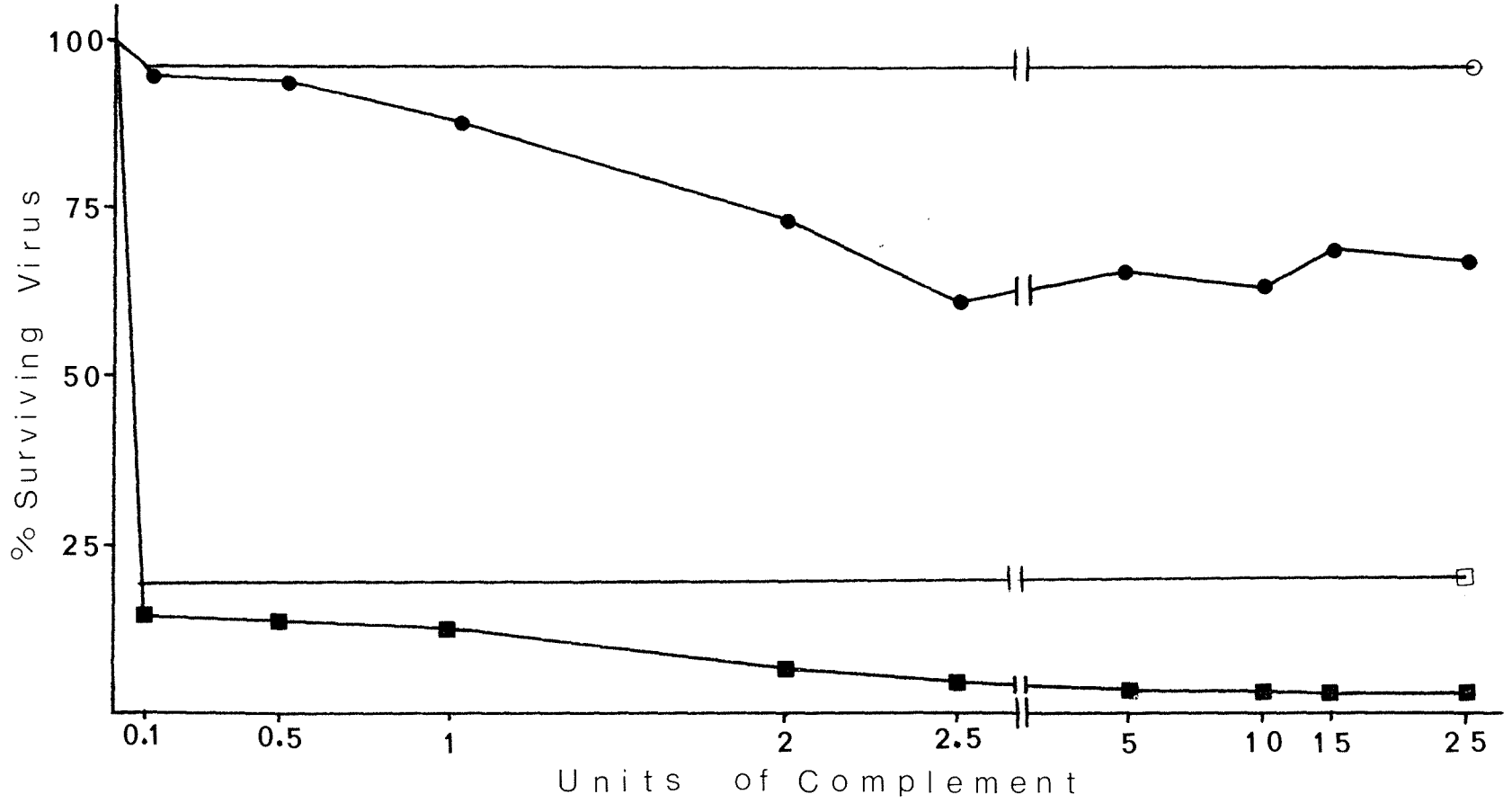
Fig. 6: The neutralization kinetic curves of early rabbit and human immune sera in the presence of varying concentrations of complement. Both sera were diluted 1/5 and surviving virus assayed after 30 min. Early human serum: ○ with heated C′ and ● unheated C′. Early rabbit serum: □ with heated C′ and ■ unheated C′.

mouse sera, taken from the 10th day post-immunization onwards, were shown to be progressively less complement requiring for neutralization. Yoshino and Taniguchi (1964) detected only CRN antibodies as early as the 3rd day after the immunization of rabbits. The statement that the first mouse serum sample similarly contained only CRN antibodies cannot be made with certainty. In this connection, it is perhaps worthwhile to mention that the virus employed in the immunization of mice was grown in BHK cells. The effect of BHK antigens on the immunization process or the presence of anti-BHK antibodies in the immune sera may have affected the neutralization of herpes virus by the mouse immune sera.

The question of the role of complement in the neutralization of viruses has been raised by many authors in the literature. Toussaint and Muschel (1962) showed that bacteriophage and antibody combined in the absence of complement but neutralization occurred on the addition of complement. Yoshino and Taniguchi (1965b) postulated two steps in the neutralization of herpes virus, the first step being irreversible binding between virus and antibody (which may still be infectious) and the second step, inactivation of virus. In the case of early immune serum containing only, or mainly CRN antibodies, the second step may be brought about by complement. The action of complement in this respect may then be similar to other antigen-antibody reactions involving complement such as cell lysis by antiserum (Oda and Puck, 1961). Berry and Almeïda (1968) showed that avian infectious bronchitis virus, in the presence of antibody and complement, developed pits in the envelope which were very similar to those present on the membranes of sensitized erythrocytes lysed by complement. This suggests that the action of complement was to cause virolysis. Wallis and Melnick (1971) have proposed that the role of complement in virus neutralization was to cause immunoaggregates.

Hamper et al. (1968; 1971) showed that 7S and 19S rabbit antibodies to herpes simplex virus from early and late sera differed in their requirement for complement for subsequent neutralization. The early 7S and 19S antibodies showed low neutralizing activity in the absence of complement and greatly enhanced neutralizing capacity on the addition of complement. In contrast, both late 7S and 19S antibodies neutralized virus to a marked degree in the absence of complement although again, neutralization was enhanced when complement was added.

The early rabbit sera used in this study seemed to contain mainly late 7S and 19S antibodies since neutralization was marked in the absence of complement.

The early human antiherpes sera appeared to contain mainly early 7S and 19S antibodies as defined by Hampar et al. (1968) since neutralization was low or negligible in the absence of complement and greatly enhanced when complement was added. These antibody types were later replaced by late 7S and 19S antibodies as the late antiserum showed a marked neutralizing potency in the absence of complement.

The experiments with the mouse immune sera showed the sequential appearance of antibodies having successively less dependence on complement for the neutralization of herpes simplex virus as immunization progressed.

Acknowledgements

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