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The Protective Effect of Different Immunoglobulins against Herpetic Encephalitis and Skin Infection in Guinea Pigs*

By

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With 9 Figures

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The elicitation of a sequential production of γM and 7 S group immunoglobulins by the infection of guinea pigs with Arboviruses (1) and the virus of foot-and-mouth disease (2) has been reported. This pattern of immunoglobulin production also has been obtained by the injection of myxovirus vaccine (3) and of bacteriophage (4). The neutralizing immunoglobulins for bacteriophage were also shown to have the different avidities indicative of the existence of these two groups of immunoglobulins (5).

Further analysis of the 7 S group with soluble protein antigens showed that the 7 Sγ1 and 7 Sγ2 immunoglobulins were differentiable not only in terms of their physico-chemical characteristics but also by their ability to produce a number of important biological reactions. Thus local and general anaphylaxis was primarily attributable to the 7 Sγ1 fraction (6), whereas the Arthus type reaction, complement fixation, the precipitin reaction and hemolysis were produced by the 7 Sγ2 fraction (7—8). The lung sensitization (9) and some passive cutaneous anaphylaxis (10) were produced primarily by the 7 Sγ1 fraction, but also could be produced by the 7 Sγ2 fraction. When guinea pigs were immunized, the initial injection caused an increase in the 7 Sγ2 fraction, but following a booster injection the increase was predominantly in the 7 Sγ1 fraction (11).

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In view of this multiplicity of roles of the immunoglobulins in biological reactions we attempted to determine their possible relationships to infection with the herpes simplex virus in guinea pigs with special reference to immune protection against this virus. The results which we here shall report appear to indicate the existence of various differences among the immunoglobulins with respect to the sequence of their appearance, their avidity in the virus neutralization reaction, the types of immunological tissue injuries which they produce and their ability to confer passive immunity.

Materials and Methods

Virus and viral antigens. The H strain of herpes simplex virus was here used exclusively. The methods for the preparation of the virus and viral antigens in rabbit kidney tissue culture cells were described previously (12—13). For immunization of guinea pigs this strain was propagated in mouse brain for 5 successive passages.

Animals and inoculation procedures. Two sizes of guinea pigs weighing 200 g and 450 g (obtained from Twin Oak Farm, Moorestown, New Jersey) were used. Intracerebral inoculation of 0.1 ml containing a suspension of $10^{5.6}$ plaque-forming units (p.f.u.) of the virus was made in the 200-g guinea pigs by insertion of the needle into the anterior fontanelle to a depth of 3 mm. Intradermal inoculation of 0.1 ml containing $10^{5.6}$ p.f.u. was made into the clipped skin of the flank or into the foot pad. For immunizing infections 0.1 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, Michigan) was added to the virus inoculum.

Preparation of specific immunoglobulin. Fifty 450 g guinea pigs were infected via the foot pad with a dose of 10^4 p.f.u. of mouse brain passage virus in the form of a 20% suspension of mouse brain. The guinea pigs were bled by cardiac puncture 1, 3, 11, 12, and 24 weeks after the infection. The sera collected at each of the above-mentioned times were pooled and stored at −20°C. The γM immunoglobulin was isolated from fresh serum by the Sephadex G-200 gel filtration method previously described (2). The 7 Sγ1 and 7 Sγ2 fractions were prepared by the procedures described by *Yagi* et al. (14). The pooled sera were dialysed at 4°C in 100 ml amounts against 20-fold their volume of 0.035 M Tris(hydroxymethyl)aminomethane (Mann Research Laboratories, New York 6, New York) — 0.005 M phosphoric acid, pH 8.5, with three changes of buffer during a period of 72 hours. Gross precipitates were removed by centrifugation at 5000 r.p.m. for 30 minutes. The supernatant fluid was adsorbed on a 3 X 40-cm DEAE-cellulose column (DE-50 Whatman, Scientifica, P.O. Box 1084, Clifton, New Jersey), which was thoroughly washed and pre-equilibrated with the above-described buffer. Gradient elution was carried out using a mixing chamber containing 800 ml of the above-described buffer, into which 400 ml of 0.5 M Tris - 0.59 M phosphoric acid buffer, pH 4.0, were introduced. The first 300 ml of eluate, which contained the 7 Sγ2 fraction and the 450th to 800th ml of eluate, which contained the 7 Sγ1 fraction were then each concentrated by negative pressure dialysis to 40 ml. After dialysis against veronal buffer ($\mu = 0.075$, pH 8.6), 20 ml of each of these preparations were placed in a 1-cm-wide longitudinal trough in the center of a 1 × 20 × 18-cm starch block, which was pre-equilibrated with the veronal buffer and subjected to electrophoresis in a zone

electrophoresis apparatus (Buchler Instruments, Inc., Fort Lee, New Jersey) at 50 volts and 5 mA for 18 hours at 4°C. The starch block was then cut into 1 cm-wide sections, which were eluted with veronal buffer. The eluates were filtered through a 0.45 micron grid Nalgene membrane (The Nalge Co. Rochester, New York) and the three eluate fractions included in the respective peaks for the 7 Sγ1 and 7 Sγ2 fractions were separately pooled and stored at −20°C.

Virus assay. The microplaque technique of *Farnham* (15) for herpes simplex virus was here used in rabbit kidney tissue cultures. Tenfold serial dilutions of the virus were prepared and 0.1 ml of each dilution was inoculated into a culture tube. After incubation at 37°C for 24 hours, plaque counts were made. A preparation of the soluble antigens of herpes simplex virus (12, 13) was heated at 56°C for 30 minutes and 0.1 ml portions of the heated material were added to tissue culture tubes which were then referred to as antigen-incorporated tissue cultures and used in the procedure which will be described later. Infected guinea pig brains were prepared as 20% suspensions

Table 1
Production of Three Immunoglobulin Herpes Neutralizing Antibodies in Guinea Pigs Infected with Herpes Simplex Virus via the Foot Pad Route

Time of serum collection after inoculation of virus (Weeks)	Herpes neutralizing antibody titers			
	Total	7Sγ1	7Sγ2	γM
1	1: 8*	1: 2	1: 2	1:6
3	1:256	1:128	1:256	1:8
11	1: 64	1: 32	1: 64	1:2
12**	1:512	1:256	1:256	1:4
24	1: 64	1: 32	1: 16	1:2

* Figure represents the value of serum pool collected from 50 guinea pigs.
** A booster injection was given a week prior to collection of serum.

in Earle's balanced salt solution, pH 7.2. The virus was titrated in 8 tubes per each 10-fold dilution and the $TCID_{50}$ determined after 3 days. The brain suspensions were extracted with ether and acetone before being used for complement fixation (CF) tests (16). The values here given for both the infectivity and CF titer are those for the 20% brain suspensions.

Neutralizing antibody titers. Twofold serial dilutions of the sera were prepared in Earle's balanced salt solution and 0.4 ml portions of these dilutions were mixed with 10^3 p.f.u. contained in 0.2 ml of virus suspension. The mixtures were incubated at 37°C for 60 minutes and 0.1 ml of each was then inoculated into each of 4 culture tubes. After thus allowing 60 minutes for adsorption of the virus by the culture, 0.1 ml of a 3-fold dilution in Earle's solution of hyperimmune guinea pig herpes antiserum with a neutralizing antibody titer of 1:256 was added to each culture to neutralize free virus. The reciprocal of the highest dilution showing a 50% reduction in the plaques in comparison with the controls was considered to be the titer of the serum. All of the sera were inactivated at 56°C for 30 minutes before being titrated.

Biological and chemical agents. The method of preparation of purified herpes serum interferon has been described previously (17). The serum obtained 6 hours

after the intravenous injection of 10^9 p.f.u. of herpes simplex virus was used unmodified as the interferon-containing serum. Peritoneal exudate and cells were obtained from guinea pigs 24 hours after their intraperitoneal infection by washing the peritoneum with 10 ml of Earle's solution and were used immediately. Purified *Escherichia coli* endotoxin was obtained from Difco Laboratories, Detroit, Michigan. Adrenalin hydrochloride solution, 1:1000 Steri-vial, was obtained from Park, Davis & Co, Detroit, Michigan, and hydrocortisone sodium succinate, Solu-cortef, from the Upjohn Co. Kalamazoo, Michigan. The 5-iodo-2′-deoxyuridine (IDU) was kindly donated by the Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

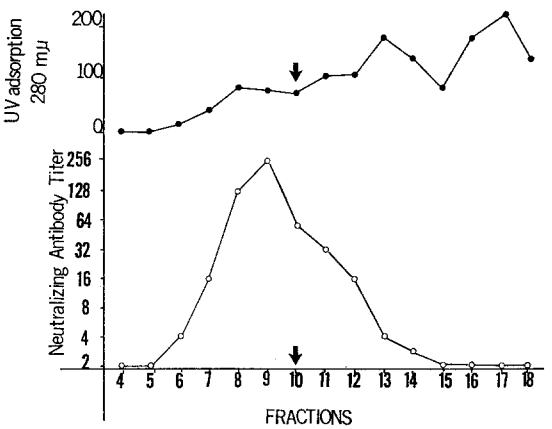

Fig. 1. Separation of guinea pig immunoglobulins by starch block electrophoresis. The arrow indicates the starting point. Peak fractions 8 –9 contained 7 Sγ 2 and 11 –12 contained 7 Sγ1 immunoglobulin anti bodies.

Results

Sequence of Immunoglobulin Production

Table 1 shows the sequence of production of three types of guinea pig immunoglobulin against herpes simplex virus following an immunizing infection via the foot pads. An early increase in serum antibodies was noted after 1 week (18), which was chiefly due to an increase in the γM fraction. In the peak period of antibody production 3 weeks after the infection the immunoglobulins consisted chiefly of the 7 Sγ1 and 7 Sγ2 fractions, which showed a 4-fold decrease 11 weeks after the infection (19). Following a booster inoculation of virus there was a marked increase in the 7 Sγ1 fraction similar to that seen with booster injections of other protein antigens (11).

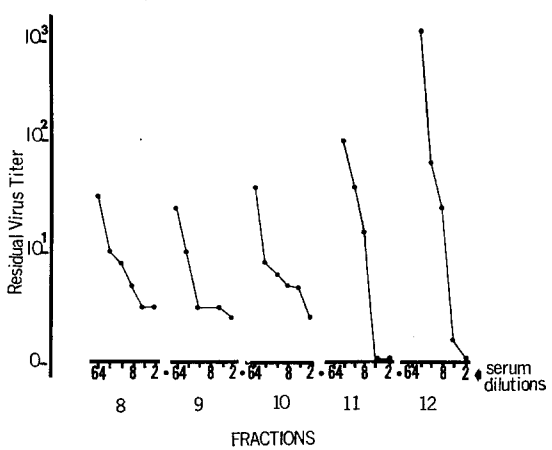

Fig. 2. Avidity differences of starch block electrophoresis fractions 8 –12 in neutralization of herpes simplex virus.

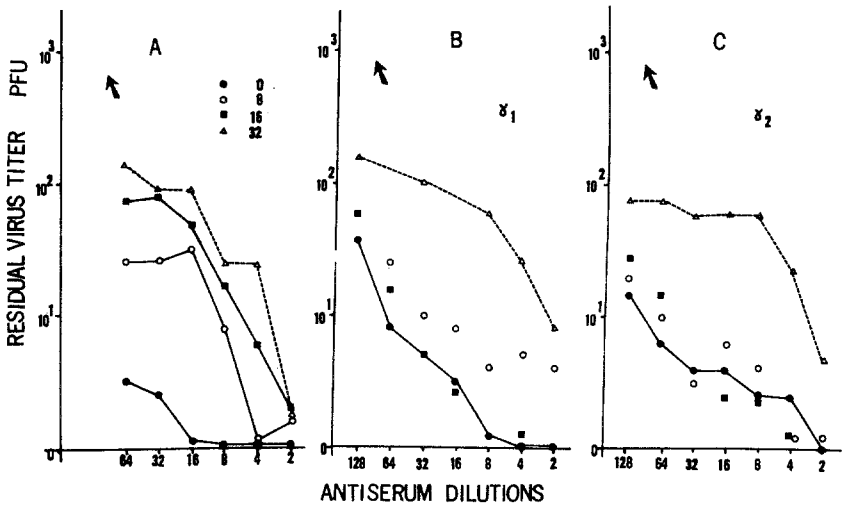

Fig. 3. Increment of non-neutralizable virus in titrations in antigen-incorporated tissue culture. The arrow indicates the original virus titer $10^{5.0}$ p.f.u. Group A shows the effect of the amounts of antigens used per tube. In CF units, ●——● = 0, ○——○ = 8, ■——■ = 16, △ --- △ = 32. Groups B and C show increment of non-neutralizable virus upon introduction of CF units, 8 (○) and 32 (△ --- △), as compared to ordinary tissue culture (■, ●——●). Group A sera were harvested at 3 weeks and Group B and C at 12 weeks.

Table 2
The Relationship of Guinea Pig Serum Herpes Neutralizing Antibody Titers to Protection against Infection with Herpes Simplex Virus in Skins

Herpes neutralizing antibody titers	Size of herpetic skin lesions after 48 hours	
	Virus alone	Virus + vasoconstriction*
Normal guinea pigs	Area in mm^3	
<1:4	94	455
<1:4	28	380
<1:4	19	345
<1:4	38	413
<1:4	38	492
<1:4	50	492
Immunized guinea pigs		
1: 16	0	492
1: 64	0	176
1: 64	0	113
1:256	0	0
1:256	0	0
1:256	0	0
1:512	0	0
1:512	0	0

* Induced by 2 μg epinephrine. Virus dose ($10^{5.6}$ p.f.u.).

Avidity of the Immunoglobulins

A pool of the sera collected 3 weeks after the infection was subjected without further processing to starch block electrophoresis and the electrophoretic fractions were tested for their herpes virus neutralizing capacity (Fig. 1). The amount of residual non-neutralizable virus was significantly different for the different fractions. Thus there was more non-neutralizable virus with the 7 Sγ2 fraction than with the 7 Sγ1 fraction (Fig. 2). However, the avidity of the 7 Sγ1 fraction was weaker in a pool of sera collected one week after a booster injection (Fig. 3). It is of interest to note that the amount of the non-neutralizable virus increased approximately 10-fold when the virus was titrated in antigen-incorporated tissue cultures. With the higher antibody titers the amounts of the non-neutralizable virus were relatively small (Fig. 3). The sera collected 1 week after the infection, which contained predominantly the γM fraction, also showed a low avidity, which is in agreement with the findings of similar studies of bacteriophage neutralization (5).

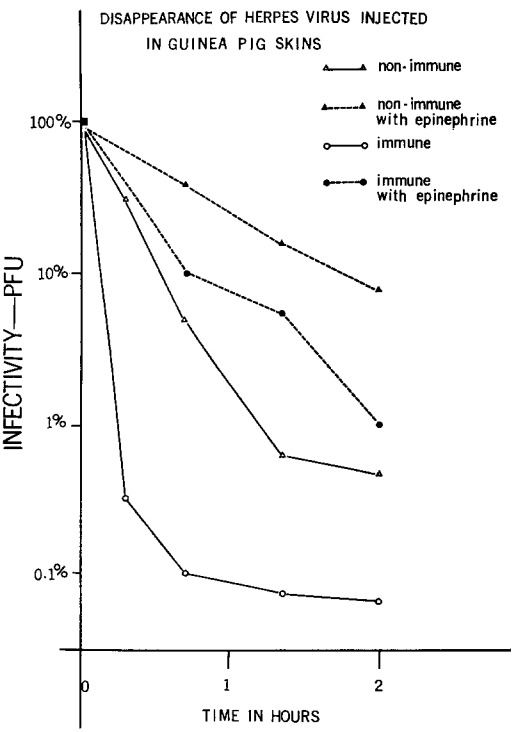

Fig. 4. The rate of disappearance of herpes virus injected in normal and immune guinea pig skins.

Local Vasoconstriction as a Limiting Factor

Since local concentration of immunoglobulins might be an important factor in the inactivation of the virus, we next determined the effect of a local vascular disturbance on the resistance of the skin to the virus ($10^{5.6}$ p.f.u. = $10^{2.6}$ minimal skin infecting dose). Table 2 shows that protection of the guinea pig skin was complete when the animal had a serum antibody titer of 1:16 or more, but that when a local vasoconstriction was produced simultaneously with the inoculation of the virus by the addition to the inoculum of 2 μg of epinephrine, those animals

having serum antibody titers of 1:64 or less were infected, whereas those with titers of 1:256 or more were protected. Fig. 4 shows the different degrees of virus disappearance in immunized animals having antibody titers of 1:128 and in normal guinea pigs in the presence and absence of local vasoconstriction. The studies made in the first 2 hours after injection of the virus showed a decrease in the virus in the immunized animals to below 10^3 p.f.u. (= 1 minimal skin infecting dose), whereas

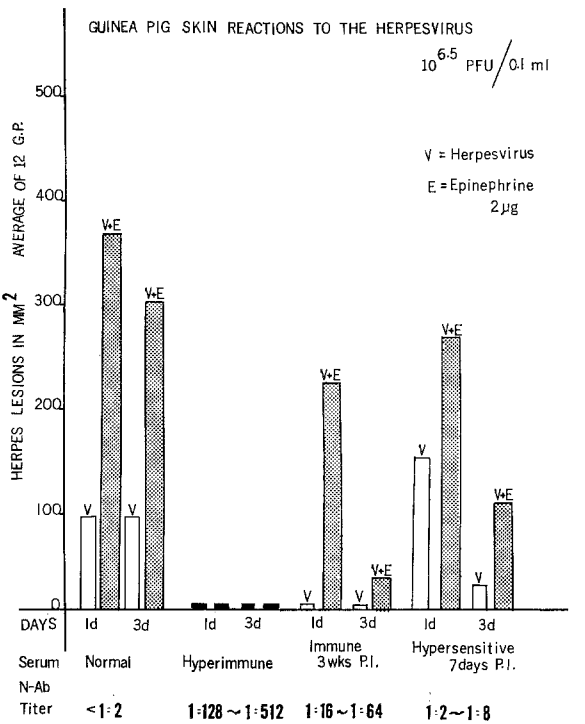

Fig. 5. Guinea pig skin reactions to herpes virus $10^{6.5}$ p.f.u./0.1 ml.

a similar decrease did not occur in the presence of a local vasoconstriction. A small portion of the virus remained viable after initial virus inactivation in a manner reminiscent of the persistence of the non-neutralizable virus in the tissue culture experiments.

Degree of Immunological Tissue Injury in the Skin

Three milliliters of the 7 Sγ1 and 7 Sγ2 fractions having neutralizing antibody titers of 1:128 (CF = 1:4) and 1:512 (CF = 1:128), respectively, were each injected intraperitoneally into 2 guinea pigs. After 24 hours, 0.1 ml of a heat-inactivated (56°C for 30 minutes) suspension of herpes

virus which originally contained 10^8 p.f.u./0.1 ml was injected intradermally into each of the guinea pigs. This virus was grown in rabbit kidney tissue cultures which contained 10% calf serum as a nutrient medium component and was washed three times in Earle's solution using ultracentrifugation at 100,000 × G for 30 minutes. Control antigen was prepared by ultrasonication of normal rabbit kidney tissue culture cells (12). An immediate type reaction at 4 hours after injection in the form

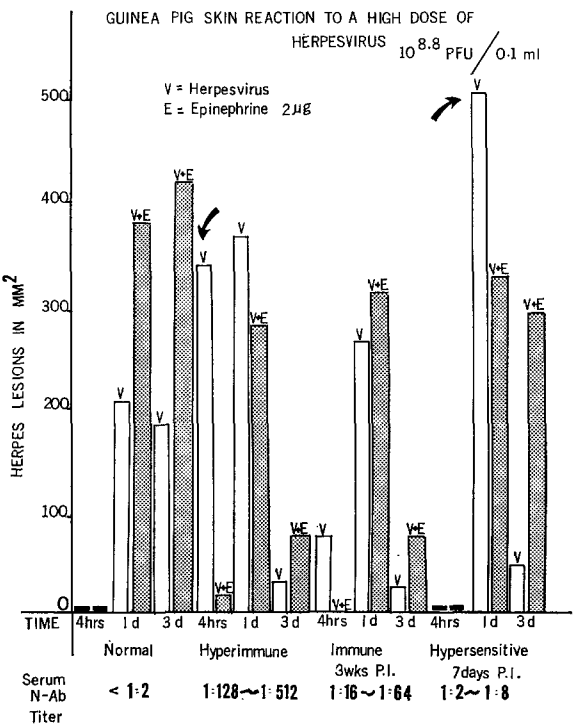

Fig. 6. Guinea pig skin reactions to a high dose of herpes virus $10^{8.8}$/0.1 ml. Serum N-Ab signifies serum neutralizing antibody. Left-hand arrow indicates the immediate type and right-hand arrow the delayed type reactions. P.I. indicates post-infection.

of erythematous and edematous lesions 12 mm in diameter was observed in the animals which had been given 7 Sγ1 fraction and hemorrhagic Arthus type lesions 10 mm in diameter were observed in the animals which had been given the 7 Sγ2 fraction against the virus antigen but not against controls. These fractions were negative in precipitin reactions and CF titers were less than 1:4 against normal components.

When active virus ($10^{6.5}$ and $10^{8.8}$ p.f.u.) was intradermally injected in normal and immune guinea pigs immunized against the 5th passage virus in mouse brain, which was free of tissue culture components, with

and without local vasoconstriction, noteworthy differences in the cutaneous lesions were observed as shown in Figs. 5 and 6. Important factors in these variations in the cutaneous lesions were the amount of the virus and the immune status of the host. As regards the skin resistance, an initial vasoconstriction prevented the immediate type cutaneous reactions, and the later cutaneous lesions were smaller than those obtained without vasoconstriction and involved a combination of erythema and necrosis. With smaller amount of virus there was a complete protection in immune

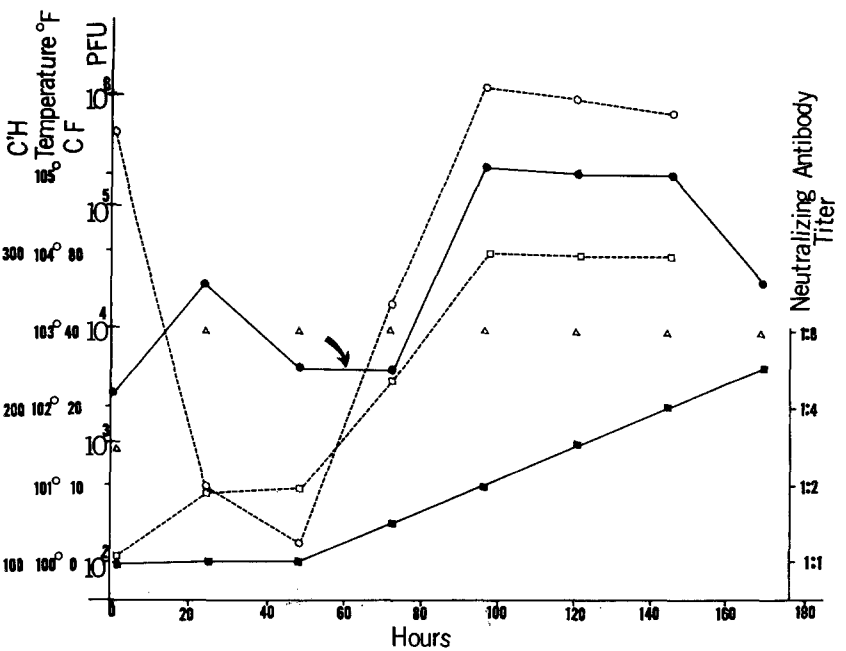

Fig. 7. The course of herpetic encephalitis in guinea pigs. The arrow indicates the point of transition from immunoglobulin-effective to ineffective stages. Virus p.f.u. (○ - - - ○), hemolytic complement titers (△), body temperature (●——●), CF antigen titers (□ - - - □) and neutralizing antibody titers (■——■).

animals. Thus when tested 7 days after the infection there was a delayed type cutaneous reaction in these animals, which was most pronounced after 24 hours and the subsequent cutaneous lesions were slightly smaller than in the controls. Normal guinea pigs showed an initial development of erythematous lesions followed by a vesiculation within 5 days, which was not seen in any of the immune animals. Two out of 24 hyperimmunized guinea pigs with mouse brain passage virus showed weakly positive precipitins against normal rabbit kidney tissue culture control antigens. However, the skin reaction to these was always less than 4 mm in diameter at 24 hours after the challenge.

The Course of Herpetic Encephalitis

The principal features in the course of the herpetic encephalitis in the guinea pigs are summarized in Fig. 7. The titer of the intracerebrally inoculated herpes virus ($10^{5.6}$ p.f.u.) decreased to 0.1% of its original value within 24 hours and remained at this level until 50 hours after the inoculation. It then increased rapidly to approximately 10,000-fold the latter titer 96 hours after the inoculation. A much higher value for this increase in titer was obtained in the antigen-incorporated tissue

Table 3. Comparative Titration of Herpes Simplex Virus in Infected Guinea Pig Brain Homogenates in Ordinary and Herpes Antigen-Incorporated Tissue Cultures

Guinea pig No. and status of brains		Ordinary tissue culture	Antigen-incorporated tissue culture	Differences
Fresh brains	1	5.47*	6.70	1.23
	2	4.70	5.88	1.18
	3	4.24	5.60	1.36
	4	4.84	6.48	1.64
	5	3.87	5.70	1.83
	average	4.62	6.07	1.45
Brains stored in 50% glycerol	1	4.00	4.70	0.30
	2	4.40	4.92	0.52
	3	4.60	5.00	0.40
	average	4.46	4.87	0.41
Fresh brains from IDU-treated guinea pigs	1	<1.70	2.30	>0.60
	2	<1.70	2.50	>0.80
	average	<1.70	2.40	>0.70

* Brains harvested 96 hours after the virus inoculation ($10^{5.6}$ p.f.u.) and the values were expressed as $\log_{10}$ of $TCID_{50}$ per ml of 20% suspension of the whole brain (32).

cultures than in ordinary tissue cultures. Thus 96 hours after the inoculation the titer was 28.2-fold higher in the antigen-incorporated tissue cultures than in the ordinary tissue cultures (Table 3). However, the difference was not so marked if the infected brains were stored in 50% glycerol at −20°C for 2 weeks. Complement fixing antigens also were detected.

The virus titer did not show any further increase after the 96th post-inoculation hour and death usually occurred within the next 6 hours.

Neutralizing antibodies usually were detectable in the serum 72 hours after the inoculation. From the difference shown in Table 3 it can be assumed that a substantial amount of neutralizing antibodies was present in the brain at an earlier stage. In rabies virus infections in mice a higher titer of the virus was found when the infected brains were homogenized

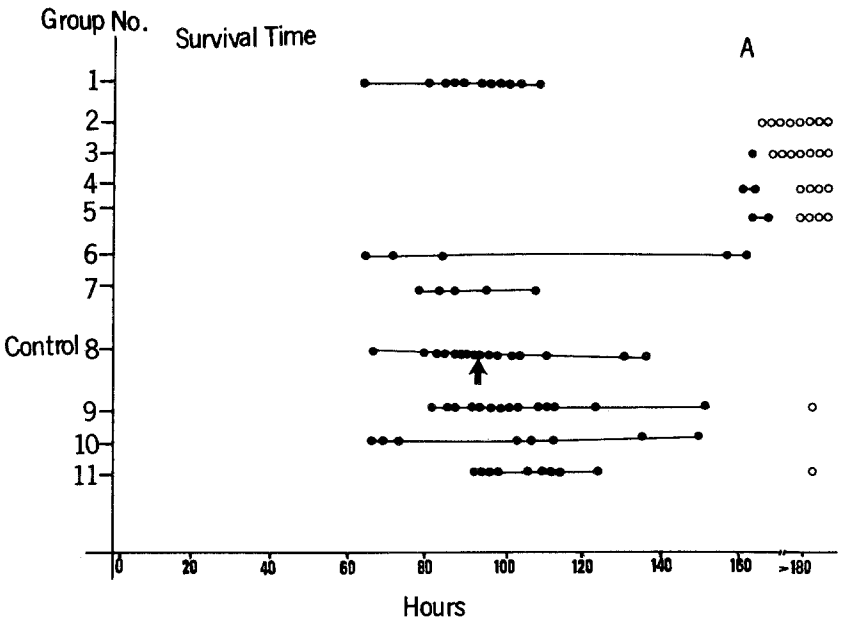

Fig. 8. Changes in survival time (ST) of guinea pigs from herpetic encephalitis (group A with virus inoculum $10^{5.6}$ p.f.u.). Each point indicates the time of death (●), or the survival (○) of a guinea pig. Group A 8 was the untreated control group and the arrow indicates the point of mean ST. The hours shown below were those after inoculation of virus.

A 1 = immunoglobulin (titer 1:128) given subcutaneously at 72 hours (ST = 96 hours).
A 2 = immunoglobulin (1:512) given at 24 hours.
A 3 = immunoglobulin (1:512) given at 60 hours.
A 4 = IDU 100 mg/kg given every 24 hours for 5 days.
A 5 = as above with addition of immunoglobulin (1:128) at 72 hours.
A 6 = hydrocortisone 120 mg/kg given every 24 hours for 5 days.
A 7 = hydrocortisone 120 mg/kg given at 72 hours.
A 8 = controls (ST = 96 hours).
A 9 = interferon-containing serum 2 ml (1024 units) given at 24 and 48 hours (ST = 101 hours).
A 10 = purified serum interferon (1024 units) given at 24 hours (ST = 105 hours).
A 11 = peritoneal exudate and cells given at 24 hours (ST = 106 hours).

with the addition of heat-inactivated virus before inoculation into ordinary assay system (20).

When specific immunoglobulins were injected intraperitoneally 60 hours after the intracerebral inoculation of virus there was complete protection (refer Fig. 8, Group A3). However, when the immunoglobulins were administered later than this (see arrow in Fig. 7) no protection by passive immunization was observable. This point in time appears

to coincide with the above-mentioned period of abrupt increase in the virus titer, which was followed by an increase in body temperature. As is shown in Table 4, the 7Sγ1, 7Sγ2 and γM fractions (the former two obtained 3 weeks and the latter 1 week after an immunizing infection) showed differing degrees of protection when administered to the guinea pigs 48 hours after their intracerebral inoculation with the virus.

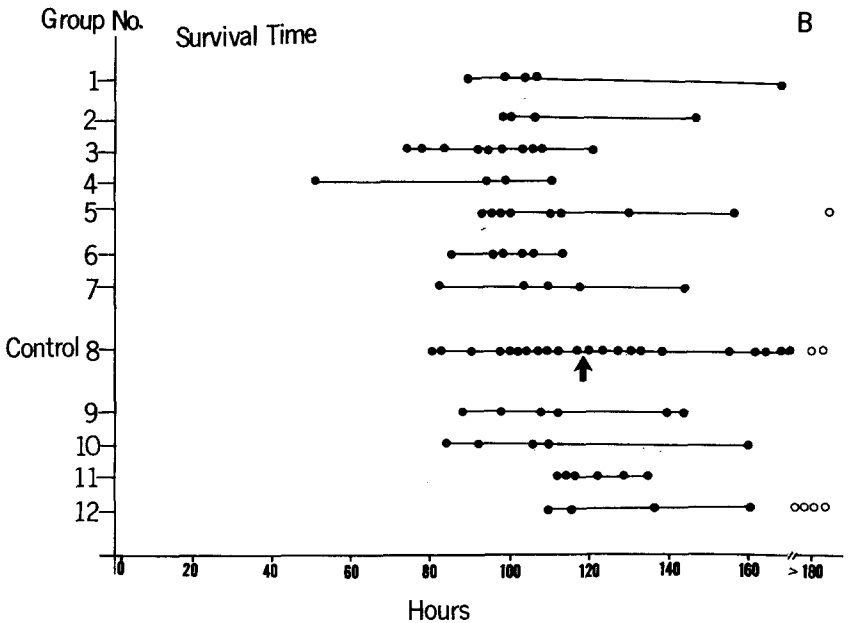

Fig. 9. Changes in survival time (group B with virus inoculum $10^{5.0}$ p.f.u.).
B1 = immunoglobulin (1:512) given at 72 hours subcutaneously.
B2 = bacterial endotoxin *E. coli* 10 μg/kg given at 96 hours, B3 at 72 hours, B4 at 48 hours and B5 at 24 hours.
B6 = injection of non-infectious herpes antigen (titer 1:128) intracerebrally 24 hours after virus (ST = 100 hours) and B7 subcutaneously at 24 hours after virus.
B8 = controls (ST = 118 hours).
B9 = intraperitoneal injection of 0.5 ml of Freund's complete adjuvant at 24 hours.
B10 = Hydrocortisone 120 mg/kg subcutaneously given at 24 hours.
B11 = IDU 100 mg/kg given subcutaneously at 24 hours (ST = 118 hours).
B12 = serum interferon (512 units) given subcutaneously every 24 hours for 5 days.

Changes in Survival Time

Figs. 8 and 9 show the time of death of the guinea pigs inoculated intracerebrally with $10^{5.6}$ p.f.u. (Group A) and $10^{5.0}$ p.f.u. (Group B) of herpes virus. Those not treated are shown as the control groups A8 and B8 with mean survival time of 96 and 118 hours, respectively. As mentioned above, almost all guinea pigs were protected when the immunoglobulin was given at 24 or 48 hours but none at 72 hours after the inoculation of the virus (Groups A1, A2 and A3). Inhibition of virus

multiplication by IDU (21) was shown by the titration results contained in Table 3. The latter agent protected 4 of 6 guinea pigs against herpetic encephalitis when given in a dose of 100 mg/kg/day during the period between 24 and 120 hours after the inoculation of the virus (Group A4). Administration of immunoglobulin 72 hours after the inoculation of the virus in addition to IDU (Group A5) did not change the survival time. A single dose of 100 mg/kg of IDU given 48 hours after the virus inoculation was without effect on the survival time (Group B11). Administration of hydrocortisone every 24 hours after inoculation of the virus (Group A6) or in a single dose 24 hours after the inoculation (Group B10) and its administration in a single dose 72 hours after the inoculation did not significantly change the survival time (Group A7). Herpes interferon given 24 and 48 hours after the inoculation in a dose of 1024 units (17) resulted in survival of only one of 15 guinea pigs (Group A9). Four of 8 guinea pigs survived when crude serum containing interferon (512 units) was given daily for 5 days and a smaller virus inoculum was used (Group B12). When 5 ml of acute peritoneal exudate and cells were given subcutaneously, there was an increase in the body temperature to above 40°C, which persisted for 48 hours, but the survival time was not significantly affected (Group A11). A protective effect of a high body temperature has previously been shown in mice infected with encephalomyocarditis virus (22). It also can be assumed that the cells in the peritoneal exudate produce interferon.

The injection of 5 ml of high neutralizing antibody titer serum (1:512) before appearance of symptoms, 72 hours after the inoculation of the virus, did not significantly affect survival time (Group B1). Shortening of survival time was reported in mice infected with Venezuelan equine encephalomyelitis virus followed by treatment with immune serum (23).

The effects of intraperitoneal injections of a non-lethal (10 μg/kg) dose of bacterial *(E. coli)* endotoxin 24, 48, 72 and 96 hours after inoculation of the virus were determined in Groups B5, B4, B3 and B2, respectively. Although the animals given the endotoxin 24 hours after the inoculation of the virus showed no effect, those receiving it at 48 and 72 hours after the virus inoculation showed a hyperreactivity to the endotoxin (24), but their survival time showed no significant deviation from the control distribution. The intraperitoneal injection of 0.5 ml of Freund's complete adjuvant 24 hours after the virus inoculation had no effect (Group B9).

The intracerebral injection of 0.1 ml of the soluble antigens of herpes simplex virus with a CF titer of 1:128, which were heated at 56°C for 30 minutes and had no infectivity in rabbit kidney tissue culture cells (12—13), 48 hours before the inoculation of the active herpes virus and their subcutaneous injection 72 hours before the inoculation produced

in each case complete protection. The intracerebral injection of the soluble antigens 24 hours after the virus inoculation (Group B6) and the subcutaneous injection of these antigens at this time had no significant effect (Group B7).

Discussion

That the protective potency of an immune serum *in vivo* is different from its potency as determined by *in vitro* serological tests has been a common experience in the case of bacterial pathogens. With a non-replicating agent such as diphtheria toxin there is a 3.3- to 6-fold difference in L_+/L_f ratios between γ- and β-immunoglobulins of diphtheria antitoxin (25).

Table 4
Protective Effects of 7 Sγ 1, 7 Sγ 2 and γM Guinea Pig Immunoglobulin Herpes Neutralizing Antibodies against Herpetic Encephalitis in Guinea Pigs

Passively transferred antibody titers	Factor of dilution	No. died/No. total		
		7Sγ1	7Sγ2	γM
512	1:1	0/6	0/6	—
256	2	0/6	0/6	—
128	4	0/6	2/6	6/6
64	8	1/6	6/6	—
32	16	3/6	6/6	—
16	32	6/6	6/6	—
None			18/18	

Note: 50% protection determined by Reed and Muench's method (32); 7Sγ1 = 1:25.3, 7Sγ2 = 1:8.32, then 7Sγ1:7Sγ2 = 3.04:1.

It has been suggested that difference in avidity may play a role in the differing degrees of protection afforded by different preparations of antiviral serum. However, evidence has been presented that immunization with poliovirus in man resulted in the production of antibodies which were widely different in avidity and titers, depending on various types of poliovirus vaccine used and the time of collection of serum after immunization (26). In addition, a case in which only γM immunoglobulin antibodies against poliovirus were produced has been described (27).

In regard to herpes simplex virus infection, the results described above indicate that differences in avidity are not as great as those in antibacterial antibodies and therefore that such differences would not play an essential role in protection, except in special cases in which the local concentration of immunoglobulin is quite low (28). The 3-fold difference between the protective titer/virus neutralizing titer ratio of the 7Sγ1 and 7Sγ2 herpes immunoglobulins (Table 4) is of the same order of magnitude

as that found in diphtheria antitoxins described above. This difference may explain some of the discrepancies in the ability of different antisera to protect guinea pigs against virus infections.

The question of why a potent immune serum fails to afford passive protection to animals infected with neurotropic viruses after a certain incubation period also has not been fully explained. Thus although it has been shown that specific immune sera can protect mice from peripheral inoculation of herpes simplex virus (29), rabbits from herpetic encephalitis (30), monkeys from paralysis produced by poliovirus (31), and mice from encephalomyocarditis virus (22), there appears to be a time after infection when immune sera cease to be effective (29).

Our present data indicate that the protective effect becomes inoperative at about the time of the beginning of the exponential multiplication of the virus following the lag phase. It appears that this critical time protection can be obtained if sufficient immune serum is present to prevent the spread of the virus from the initially infected cells.

Summary

The relationships of the γM, 7 Sγ1 and 7 Sγ2 immunoglobulins to the pathogenesis of herpetic skin infections and encephalitis were investigated in guinea pigs. The differences in the sequential appearance, avidity and secondary response of these immunoglobulins to booster inoculations of viral antigen and the types of immunological tissue injuries in immunized animals were detected. When the immunoglobulins were purified by chromatographic and electrophoretic techniques there was a 3-fold difference between the passive protective potencies of the 7 Sγ1 and 7 Sγ2 immunoglobulins. However, these immunoglobulins could protect guinea pigs only when they were given prior to the phase of exponential multiplication of the virus.

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