TABLE I.

Cross Neutralization of the Pseudorabies and "Mad Itch" Viruses.

Subcutaneous injection of 1.5 cc. containing 1 cc. of virus (10% suspension of virus-containing rabbit brain)	0.5 cc. serum† swine	Guinea Pig No.	Result
Pseudorabies	673—normal	506	Died—71 hr.—control
"	" "	507	" 80 " "
"	810 "	514	" 68 " "
"	" "	517	" 66 " "
"	" "	543	" 63 " "
"	1235—pseudorabies convalescent	500	No illness
"	" " "	501	" "
"	" " "	502	" "
"	" " "	503	" "
"	1185 " "	494	" "
"	" " "	495	" "
"	772—"mad itch" convalescent	522	" "
"	" " " "	524	" "
"	" " " "	542	" "
"	" " " "	546	" "
"Mad itch"	673—normal	504	Died—73 hr.—control
" "	" "	505	" 90 " "
" "	810 "	515	" 105 " "
" "	" "	516	" 72 " "
" "	" "	547	" 77 " "
" "	1235—pseudorabies convalescent	496	No illness
" "	" " "	497	" "
" "	" " "	498	" "
" "	" " "	499	" "
" "	1185 " "	526	" "
" "	" " "	527	" "
" "	772—"mad itch" convalescent	518	" "
" "	" " " "	521	" "
" "	" " " "	544	" "
" "	" " " "	545	" "

† The serum and virus were mixed and stored in the refrigerator over night (17 hours) prior to inoculation into the test guinea pigs.

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Herpes Antiviral Substances; Distribution in Various Age Groups and Apparent Absence in Individuals Susceptible to Poliomyelitis.

ELLIOTT R. WEYER. (Introduced by Simon Flexner.)

From the Rockefeller Institute for Medical Research.

That the virus of herpes simplex of human origin is capable of producing neurotropic changes in the rabbit and certain other rodents was shown by Doerr[1] and others. Since the work of Zinsser

[1] Doerr, R., *Z. f. Haut- u. Geschl.*, 1924, **18**, 27.

and Tang,[2] Andrewes and Carmichael[3] it has been known that the blood serum of many individuals contains an antibody which may cause *in vitro* neutralization of this virus so that the mixture becomes non-pathogenic for these animals. Examinations of human sera for herpes antibody have for the most part been made to trace some connection between epidemic encephalitis and herpetic infection, symptoms and pathology of the latter when produced in rodents showing some similarity to the former disease in man. The results of such tests have been negative, convalescent encephalitis patients having approximately the same antibody incidence as is found in "normal" individuals.

Brain[4] showed fairly definitely that an attack of herpes in man always gives rise to the production of more or less permanent blood-born antibodies though these antibodies give no apparent protection against future attacks. Because the white mouse is susceptible to herpes virus when introduced intracranially (Andervont[5]) it has been possible to study conveniently the action of neutralizing serums with a fair degree of accuracy. The availability of a large series of human sera from known age groups suggested the desirability of studying herpes virus neutralizing capacity at various ages.

To determine the ability of a serum to neutralize, equal volumes of centrifugalized 5% emulsion of virus-containing mouse brain and the serum under investigation were mixed and allowed to remain at room temperature for about an hour (time unimportant) before intracranially inoculating two 18-20 gm. white mice under light ether anesthesia. In order that the virus material be of as uniform potency as possible throughout the tests brains were used only from mice which had died during the fourth or fifth day after inoculation. The volume of inoculum was invariably 0.05 cc. to each mouse and duplicate inoculations were made throughout. Control mice received 0.05 cc. of a 2.5% and a 0.5% suspension, the former representing the same amount of virus received by the test mice while the latter dilution served to give an index of the potency of the virus through the delay in symptoms caused by this further dilution. As all mice receiving the 0.5% virus died, the test mice were injected with a minimum of 5 killing doses of virus, either neutralized or not depending upon the serum in the mixture. However, accurate

[2] Zinsser and Tang, *J. Immunol.*, 1929, **17**, 343.

[3] Andrewes and Carmichael, *Lancet*, 1930, **1**, 857.

[4] Brain, *Brit. J. Exp. Path.*, 1932, **13**, 166.

[5] Andervont, *J. Inf. Dis.*, 1931, **49**, 507.

titrations of the virus at frequent intervals showed that the mice were in reality receiving upward of 50 killing doses.

In general, the mice which received the serum-virus mixtures either died about the fifth day, or remained well. Occasionally the symptoms were retarded in development but this was an exception, and if the illness did not become apparent until after the tenth day the result was considered positive for neutralization. Such mice usually lapsed into a comatose state in which they lingered for as long as 2 weeks. Another form of discrepancy occasionally met was that one mouse of a pair died promptly while the other failed to die but showed symptoms with ultimate improvement. In this event the serum was usually retested and in general the result was such that we may assume either that the surviving mouse had unusual individual resistance to the virus or that the inoculation had not been properly made.

The results have been tabulated according to the ages of the individual donors in groups of a 5-year interval. Above the age of 45 the sera were few and were grouped together.

TABLE I.

Age Group	No. Sera Tested	% Neutralizing
0-5	28	14
5-10	26	38
10-15	11	40
15-20	19	90
20-25	28	90
25-30	14	71
30-35	22	68
35-40	16	75
40-45	10	65
45 and over	13	54

These results are quite in harmony with those of Zinsser and Tang,[2] who reported neutralization in 25% of sera from children below the age of 7 years, 43% in "children in general" and what would appear to be 64% neutralization in adults if we may strike an average on his statistics dealing with "normal" adult and convalescent encephalitic adult sera.

The sera upon which our observations were made came from several geographical sources, eastern United States, the West Indies, and China. There is nothing to indicate that geography plays any appreciable part in the reactions.

One would, of course, assume that the antibody develops in the individual as a response to herpetic infection. In view of the ubiquity of herpes simplex it would be extremely difficult to exclude

the rôle of the virus. Friedberger *et al.*[6] similarly finding in human sera "antibodies" capable of serological reactions with sheep and rabbit erythrocytes came to the conclusion that these substances are non-specific in the serum and express processes which arise physiologically during the maturation of the individual. Their figures indicate that such antibodies are not found in the blood of a child at birth but that they appear as the age of the individual increases so that 90% of the sera from persons above 10 years show a positive reaction despite the fact that no parenteral contact with sheep or rabbit antigens would normally be supposed. Coca's work[7] on the diffusibility of native proteins perhaps weakens this argument for the natural acquisition of antibodies as a feature of maturation. That a similar curve has been observed through the age groups with respect to the antitoxins for diphtheria and scarlet fever need scarcely be mentioned except that the available figures show that the same curve obtains in geographical locations from which these diseases are absent.

Of the sera available for examination from the standpoint of herpes antibody as reported above, 27 were specimens drawn from children who proved to have been susceptible to poliomyelitis, all of them victims of the 1931 epidemic. The bleedings were in most cases "initial" ones, *i. e.*, they were made upon diagnosis of poliomyelitis for the purpose of neutralization tests with the virus of poliomyelitis. The results of the tests with herpes virus showed them to be individually and collectively devoid of any neutralizing effect. A comparison of the results with the incidence for herpes virus neutralization in the same normal age groups gives:

TABLE II.

Age Group	No. sera, non-polio. patients	% neutralizing herpes virus	No. sera, polio. patients	% neutralizing herpes virus
0-5	16	17	12	0
5-10	18	50	8	0
10-15	9	55	2	0
Age?	—	—	5	0

The neutralization tests upon the poliomyelitis sera were repeated twice using a minimum of 6 test mice upon each serum. This absence of herpes antibody among persons subject to poliomyelitis suggested the possibility that either the absence of herpes virucidal bodies was indicative of the absence of poliomyelitis antibody, or vice versa, or, it might show that a single "panimmune body" was

[6] Friedberger, *et al.*, *Z. f. Imm.*, 1929, **64**, 294.

[7] Coca, *J. Imm.*, 1930, **19**, 405.

lacking from the serum which if present would accomplish the neutralization of either or both viruses.

In previous experiments the blood serum from several animal species, viz., hen, rabbit, mouse, guinea pig, sheep, horse and monkey was tested for a herpes neutralizing factor. A *Macaccus rhesus,* the serum from which caused a 5-day delay in the appearance of herpetic symptoms in test mice, was the only animal in this instance which gave any evidence of possessing virucidal antibodies. This monkey was a convalescent poliomyelitis animal which had recovered from a mild attack of experimentally induced poliomyelitis 6 months previous to the time of the bleeding tested. The result led to the testing of 4 additional monkeys; one normal animal gave no trace of neutralization. The second, a convalescent which had been further "reinforced" by the inoculation of additional virus after recovery which gave complete neutralization. The third, a convalescent from the 1931 virus, which also gave complete neutralization. The fourth monkey had been given poliomyelitis virus intradermally but at no time had it shown paralytic symptoms. The serum did not neutralize the herpes virus.

The normal monkey control in the group just described was then given 5% herpes virus intradermally over a period of 3 weeks, 10 cc. of suspension being used in all. The serum now caused a retardation in the herpetic symptoms in test mice of from 4 to 5 days. Difficulty has been experienced in producing a high titre serum not only in the non-susceptible monkey but also in the highly susceptible rabbit and mouse in absence of infection. Recovery from infection in the latter animals, however, produces a serum of considerable potency. The most potent anti-herpes monkey serum came from an animal which had not received herpes virus but which had acquired poliomyelitis antibodies through an experimental poliomyelitis infection.

Conclusions. An antiviral substance which has the ability to neutralize the virus of herpes simplex has been found to be present in the blood early in the life of a few individuals. As age advances, though not necessarily because of maturation, more individuals acquire the antiviral property. A peak is reached in early adult life, after which apparently a decline sets in.

A group of sera from children whom we may assume carried no antiviral substances for poliomyelitic infection were shown likewise to be devoid of antiviral substances toward herpetic infection. Finally, herpes virus neutralizing properties have been discovered in the blood of monkeys which have reacted to poliomyelitis virus.