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The Precipitation of Latent *Herpes simplex* Encephalitis by Anaphylactic Shock.*

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In a preliminary study[1] we found that anaphylactic shock caused the precipitation of *Herpes simplex* encephalomyelitis from the latent state in a rabbit. The results here reported represent subsequent studies of this phenomenon.

It has long been recognized that Herpes infection in man is frequently recurrent in character and that exacerbations may be produced by a number of stimuli including upper respiratory infections, fever, emotional stress, menstruation, mechanical irritation, heat, exposure to ultra-violet light, and digestive disturbances. The general censensus is that these diverse stimuli activate a latent infection.[2] Levaditi, Harvier, and Nicolau[3] demonstrated Herpes virus in the saliva of normal people. Flexner and Amoss[4] obtained this virus from the cerebrospinal fluid of a luetic patient who was not suffering from Herpes and who gave no history of recent herpetic infection. Bastai and Busacca[5] found Herpes in the cerebrospinal fluid between infections in patients who were subject to recurrent cold sores. Perdrau[6,7] presented data which might be interpreted as evidence of latent Herpes simplex infection in rabbits when he showed that isolation of this virus from the brains of fatal human cases of *Encephalitis lethargica* could be facilitated by mixing the human brain suspensions with brain suspensions of rabbits actively immunized against Herpes. Da Fano and Perdrau[8] and Perdrau[7,9] showed that occasionally herpetic encephalomyelitis in rabbits exhibits a prolonged smouldering course or shows sudden spontaneous exacerbations as long as 6 months after inoculation. Perdrau[7] showed that this indolent type of disease of rabbits could be produced with increased frequency if the inoculated rabbits had first been partially immunized against Herpes.

That the capacity to produce latent infection is not a peculiarity of the Herpes virus has been shown by a large number of clinical and experimental observations demonstrating that many viruses possess such properties. Traub,[10] Skoglund and Baker,[11] MacCallum and Findlay,[12] Traub[13] and Leichenger, Milzer, and Lack,[14] have shown that the virus of lymphocytic choriomeningitis may have latent and intermittently active phases in both animals and man. Similarly, latent infections have been demonstrated to exist with a large number of viruses: infectious anemia virus of horses,[15] foot and mouth disease virus

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in cattle,[16] viruses of rinderpest in cattle, hogcholera in swine, fowl pox and laryngotracheitis in hens,[17] Theiler's encephalomyelitis virus in mice,[18] psittacosis virus in birds,[19,20,21] mouse pneumonitis virus,[22,23] pneumonia virus of mice,[24,25] St. Louis encephalitis virus in mice,[26] parotid gland virus of guinea pigs,[27] vaccinia virus in rabbits,[28,29] and perhaps mammary cancer virus in mice.[30,31,32] Furthermore, it may be as Andrewes[2] suggests, that the antibody response to some viruses such as influenza, yellow fever, and poliomyelitis in the absence of clinical disease is due to temporary latent infections.

Kirschbaum[33] and Hoff and Silberstein[34] perhaps by activating a latent infection produced a transmissible encephalitis in dogs by hepatic artery ligation or Eck fistula production. Perdrau[9] employing a number of unrelated devices including subcutaneous guanadine-HCl injections, local brain trauma, and injection of large amounts of inactivated virus was unable to activate latent Herpes and Vaccinia infections, but succeeded in producing the symptomatology and typical pathology of Borna disease in rabbits 18 months after the original infection. Andrewes and Miller,[35] and Rivers and Tillet[36,37] were able to precipitate apparently latent virus III testicular infections in rabbits. McIntosh[38] was able to precipitate sarcoma virus infection by injecting tar into the connective tissue of infected animals. In plants, Bawden,[39] Grainger,[40] Best,[41] Walker and Larson,[42] Shapovalov and Lesley[43] have shown that manipulation of environmental factors, particularly temperature and light intensity, can bring about activation of latent virus infections. Traub[13] has precipitated lymphocytic choriomeningitis infection from the latent state in mice by injecting nutrient broth intracerebrally and Shope has precipitated latent influenza virus infection in swine.[45–49]

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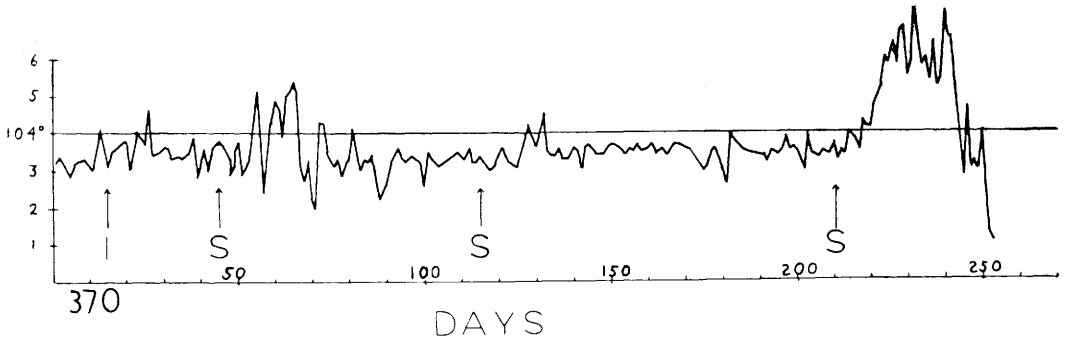

FIG. 1.

Temperature curve of Rabbit No. 370. Three instances of precipitation of *Herpes simplex* infection following anaphylactic shock. I—inoculation of H.F. virus. S—administration of anaphylactic shock. *—virus recovered 9 months after initial infection.

Materials and Methods. This experiment was performed on young adult albino rabbits which were sensitized by the administration of egg white on alternate days in the following dosages: 1 cc i.v.; .5 cc i.v.; 1 cc i.m. The rabbits were then inoculated into the quadriceps muscle group with .5-1 cc of a 10^{-1} suspension of mouse brain from mice which had died with *Herpes simplex* encephalitis.

Inoculation of rabbits in this way produces 5 courses of disease. 1. Somewhat less than 50% of inoculated rabbits develop fever, acute ascending myelitis; encephalitis, meningitis and death in a 3 to 4 week period.

2. Some rabbits develop acute disease with high fever, myelitis, encephalitis, and meningitis, but survive and apparently completely recover from the infection.

3. Some develop a mild form of the disease with minimal symptoms of encephalomyelitis and early recovery.

4. A few develop chronic, smouldering progressive encephalomyelitis with lowgrade fever and flare-ups of increased activity.

5. Some rabbits show no demonstrable infection.

From a large group of rabbits inoculated into the quadriceps muscle as described those showing courses 2, 3, 5 were selected for further study. Daily temperatures were recorded and each rabbit was checked regularly for the presence of symptoms. After evidence of the acute infection had disappeared and the rabbits had been well for from 1 to 3 months each was subjected to anaphylactic shock by

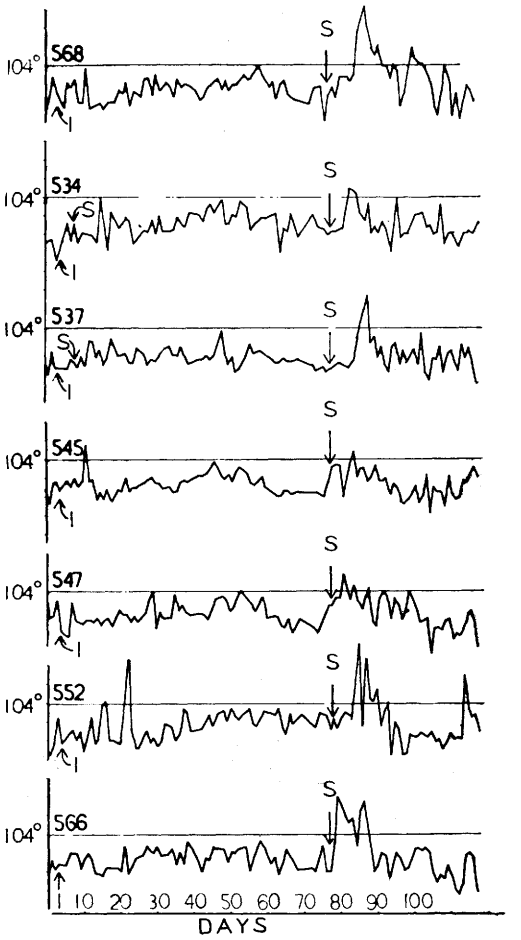

FIG. 2.

Temperature curves from 6 rabbits. Each curve shows an instance of precipitation of latent Herpes infection following anaphylactic shock. I—inoculation with *Herpes simplex* virus. S—administration of anaphylactic shock.

TABLE I.
Precipitation of Latent *Herpes simplex* Infection in Rabbits.

No.	No. of precipitations	Date of original virus inoc.	Date of onset of precipitation	Degree of shock	Incubation period days	Fever to	Manifestion of disease
531	1	10-1-46	2- 1-47	mild	5	105.4	encephalitis, myelitis, paralysis, death.
534	2	"	12-23-46	"	5	104.4	" meningitis.
			2- 1-47	"	6	105.2	" "
537	1	"	12-26-46	moderate	8	105.8	"
545	1	"	12-23-46	mild	5	104.4	"
547	1	"	12-21-46	moderate	3	104.9	" meningitis, myelitis, death.
548	1	"	1-22-47	"	2		
552	1	"	12-24-46	mild	5	106.4	" "
562	1	"	1-31-47	no apparent shock	6	107.2	" prolonged illness.
563	1	"	1-30-47	mild	7	105.3	" meningitis.
568	1	"	12-25-46	"	7	107.2	" myelitis
569	1	"	12-22-46	"	5	106.4	" " prolonged C.N.S. illness, death.
566	1	"	12-19-46	"	2	106.0	signs of encephalitis.
679	1	12-18-46	5- 1-47	no apparent shock	14	104.8	encephalitis, myelitis.
681	1	"	4-22-47	mild	6	105.4	" death.
370	3	12-22-45	2- 7-46	moderate	9	104.6	" myelitis, meningitis.
			4-22-46	mild	7	104.6	"
			7-10-46	severe	8	107.4	" myelitis, death.
126	1	7-30-44	2-13-45	"	5	106.2	" meningitis, death.

the intravenous injection of .2 to .6 cc of egg white. The ensuing shocks were graded mild, moderate, or severe.

Results. Fig. 1 shows the temperature curve of an illustrative animal.

Fig. 2 shows the temperature curves of a representative series.

The findings of these experiments are summarized in Tables I and II. Encephalomyelitides were precipitated from the latent state 19 times in 16 rabbits. The symptoms of encephalitis included disorientation, gnashing of teeth, nystagmus, muscular twitchings, drooling of saliva, and vestibular fits. Opisthotony was considered to be evidence of meningitis and flaccid paralysis and diminished or absent deep reflexes in the extremities as evidence of myelitis. The anaphylactic shocks were not followed immediately by activity of the infection, but in each case an incubation period varying between 2 and 14 days separated the anaphylactic shock from the exacerbation. The mean incubation period

TABLE II.
Incidence of Precipitation of *Herpes simplex* Encephalitis Following Anaphylactic Shock.

1.	No. of rabbits with possible latent infection studied	44
2.	No. of anaphylactic shocks administered	52
3.	No. of rabbits showing the precipitation phenomenon	16
4.	No. of precipitation infections noted	19
5.	No. of apparently spontaneous exacerbations noted during period of study	4
6.	No. of times anaphylactic shock failed to induce precipitation of *Herpes simplex*	28
7.	No. of rabbits showing questionable precipitation following anaphylactic shock	5

was 6 days and the most commonly occurring one was 5 days. The spontaneous and induced exacerbations were symptomatologically alike, and in fatal cases from both groups virus was recovered from the central nervous system which was identical to the Herpes virus inoculated several months previously. In the 5 instances described as questionable precipitations in Table II the active infection was too far removed from the anaphylactic shock to be unequivocally attributed to it in 2 cases and the evidence of activity was questionable in 3.

Table I contains some evidence that the severity of the shock shows little correlation with the incidence of activation of latent infection. According to the criteria used 2 rabbits demonstrating no shock following reinjection of egg white showed the precipitation phenomenon and several showed successful activation with mild shock.

In order to compare the incidence of precipitation due to anaphylactic shock with that of spontaneous activation the following formulae were used.

$$I_1 = \frac{P}{T_1} = \frac{19}{728} = \frac{26.4}{1000} \text{ rabbit days of exposure}$$

$$I_2 = \frac{S}{T_2} = \frac{4}{7934} = \frac{.5}{1000} \text{ rabbit days of exposure}$$

Where I_1 = incidence of precipitation infections, P = number of precipitation infections, T_1 = number of anaphylactic shocks administered × 14 (arbitrary number of days of anaphylactic shock influence), I_2 = incidence of spontaneous infection, S = number of spontaneous infections; T_2 = total rabbit days of the experiment —T_1.

Herpes simplex virus was recovered from the central nervous system of rabbits of this series following fatal precipitation infection, during periods of quiescence after precipitations, following fatal spontaneous exacerbations, and during chronic smouldering encephalitis. At autopsy the brains and spinal cords of the rabbits were removed under aseptic precautions, placed in 50% glycerine in normal saline buffered to pH 7.4, and stored in the icebox from 3 days to 3 weeks. Pools were made of the central nervous tissues of each rabbit, ground with mortar and pestle with normal saline to prepare a 10% suspension of rabbit brain and inoculated intracerebrally into young adult mice. In the cases in which the mice developed fatal encephalitis several passages through mice were accomplished and then further studies were carried out to identify the virus by its biological and immunological properties. These studies included titration in mice, corneal inoculation in rabbits, pathological study of brains of corneally infected rabbits and neutralization studies with immune rabbit serum and pooled human serum.

Discussion. These data raise questions concerning the nature of the disease observed during the periods of exacerbation. Is it a renewal of the same disease which was re-

sponsible for the original illness, or without the renewal of the activity of the infectious agent does the anaphylactic shock merely activate old lesions? Is the exacerbation a true herpetic encephalitis, or is it merely a flare-up of an old inflammatory locus due to some non-specific stimulus? The evidence available at the moment indicates that we are dealing with a renewal of the essential disease process. From the point of view of the symptomatology, it may be observed that the exacerbations frequently show more severe and more widespread evidence of disease than do the initial infections. This would indicate enlargement of the affected area in the nervous system. Even more convincing is the evidence already reported[44] of recovery of *Herpes simplex* virus from the nervous system of these rabbits as long as 9 months after the initial infection. The virus is to be found in the nervous system not only in the active stages of the disease, but also during quiescent remissions. To interpret these results in terms of the relationship of antigen-antibody reactions to the dynamics of infection is speculative, but hypotheses based on this line of thinking are intriguing. It seems that these rabbits have become carriers by overcoming the initial infection yet continuing to harbor virus in their tissues. That anaphylactic shock results in a breakdown of this symbiosis in which the virus is present in the central nervous system without signs of disease may indicate that immunity to the virus suffers a temporary lapse during or following the anaphylactic shock which allows the virus once again to express its virulence. To claim that anaphylactic shock is in any way a specific means of precipitating latent neurotropic virus infection is not intended, but whatever might be the alteration in the immune or physiologic state of the animals which is responsible for disruption of the established host-parasite balance, it is effectively produced by anaphylactic shock. Although the recently recognized encephalitis following pertussis immunization shows a striking parallelism to this phenomenon, we are unaware of the demonstration of identical disturbances in recognized human disease. Careful elucidations of the factors involved in the largely unexplored realm of post-infectious and undiagnosed encephalitides commonly occurring in childhood, and in exacerbations of symptomatology in the post-encephalitic patients might reveal mechanisms in common with the experimental disease.

Summary. 1. Evidence that virus disease may develop a latent state is reviewed with an account of the reports of successful activations of latent infections.

2. Nineteen instances of precipitation of latent herpetic infection in rabbits by anaphylactic shock are presented.

3. Recovery of *Herpes simplex* virus from rabbit central nervous system during periods of (a) quiescence, (b) chronic encephalitis, (c) spontaneous exacerbations, and (d) anaphylactically induced precipitations of encephalitis is reported.

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Invasion by *Shigella sonnei* of Tissues of Mice Following Gavage with Viable Shigella.*

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In a previous publication[1] we reported that white mice did not show any signs of infection but did develop a considerable degree of active immunity against intraabdominal chal-

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