THE THERMAL INACTIVATION TIME AT 41.5°C. OF THREE STRAINS OF HERPES SIMPLEX VIRUS*

By RUTH A. BOAK, Ph.D., CHARLES M. CARPENTER, M.D., and STAFFORD L. WARREN, M.D.

(From the Division of Radiology, Department of Medicine of the University of Rochester School of Medicine and Dentistry, Rochester, New York)

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The preceding communication (1) reports the recovery of 4 strains of a filter-passing virus from symptomatic herpes in patients treated with artificially induced fever, and describes their immunological relationship to a known (Frank) strain of herpes virus. The appearance of herpes after long fever treatments indicated that the virus was relatively thermostable at a temperature of 41.5°C. This study was undertaken to determine the resistance of the virus to 41.5°C. *in vitro*. The thermal inactivation time was based upon the length of exposure required to inactivate the virus so that it produced neither keratitis nor encephalitis in rabbits.

The thermal death time of several species of bacteria has been determined at fever temperatures (2–4), but we have succeeded in finding only a few references in the literature to the resistance of viruses to such temperatures. It was our observation that treatment with a single 5 hour fever at 41.5°C. failed to cure rabbits injected intracerebrally with herpes virus. Kopeloff and Holden (5) observed that a series of fever treatments did not prevent the development of encephalitis in rabbits that had been inoculated with the El Perdrau or Frank strains of herpes virus, and furthermore, that heating of the El Perdrau strain before injection did not inactivate it sufficiently to prevent the development of the disease. Fever temperatures likewise had no apparent injurious effect on the virus of poliomyelitis in monkeys, as reported by Jungeblut and Kopeloff (6), or on the viruses of infectious myxomatosis and Shope's fibroma in rabbits, as observed by McKinley and Acree (7). Thompson (8), on the other hand, observed that elevating the skin temperature of rabbits to approximately their normal rectal temperature (39–40°C.) inhibited the development of tumors in the skin by the fibroma virus. He also stated that myxoma virus was "definitely influenced" by fever therapy.

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The following observations were made on the thermal inactivation time at 41.5°C. of 3 strains of herpes virus. 2, H.F.[1] and Frank,[1] were standard strains, the third, Go, had been recovered recently from the vesicle fluid of a patient who had developed symptomatic herpes after fever treatment for gonococcal infection.

Procedure

Intracerebral serial passage from rabbit to rabbit was made with each of the viruses. Moribund animals showing symptoms of herpes encephalitis were etherized and killed, and the brain was then removed aseptically. Aerobic and anaerobic cultures were made to establish freedom from bacterial infection. A 20 per cent brain suspension was prepared by grinding 1 part of cortical substance with 4 parts of a buffered physiological salt solution in a sterile mortar. With a sterile syringe, 1 cc. of each suspension was transferred to long stemmed sterile glass vials with a capacity of 2 cc. The stem of the vial was sealed in a gas flame, and groups of each were immersed in a water bath set at 41.5°C. and in a control bath at 37°C.

Preliminary observations had shown that the thermal inactivation time at 41.5°C. of the Frank and H. F. strains of herpes virus was approximately 80 hours. In this experiment, therefore, examinations for viability of the viruses were made at 10 hour intervals for a period of 100 hours. The control suspensions in the 37°C. bath were tested for viability at the beginning and end of the experiment. The method was as follows. A vial was removed from the bath and shaken, the seal was broken, and 0.3 cc. of the contents was withdrawn into a sterile syringe and then injected intracerebrally into young, ether-anesthetized, blue rabbits weighing from 1,000 to 2,000 gm. 0.05 cc. of the suspension was also placed on the right cornea, which had first been prepared by the application of a 1 per cent solution of cocaine and then scarified. The rabbits were placed in individual cages. Observations were made at frequent intervals and the temperature was taken daily. As soon as death occurred, an autopsy was performed. The brain was removed and cultured; a cross-section was fixed and stained by Giemsa's method and later examined for intranuclear inclusion bodies. Another section was preserved in a neutral 50 per cent glycerine solution.

RESULTS

The Frank and H. F. strains of herpes simplex virus produced a fatal encephalitis in rabbits after exposure to a temperature of 41.5°C. for 70 hours. The suspensions heated for 80 hours or longer, however, did not cause disease. Strain Go lost some of its infectivity for rabbits after 20 hours of heating. Suspensions heated for 30 and 40 hours produced a marked keratitis and symptoms of encephalitis, such as fever, hyperkinesia, and twitching of the head, from which the animals recovered within 3 weeks. A rabbit injected with suspension heated for 30 hours showed the

[1] Obtained from The Rockefeller Institute for Medical Research through the courtesy of Dr. Peter K. Olitsky.

more severe symptoms. The temperature of the animal rose to 41.5°C. on the 11th and 12th days after inoculation, while the temperature of the rabbit receiving the 40 hour suspension was elevated for only 6 days and reached a maximum height of 40.2°C.

The dermotropic factor of the H. F. strain appeared to be more sensitive to heating than the neurotropic factor. The virus no longer infected the cornea after it had been heated for 50 hours at 41.5°C., but it continued to

TABLE I

The Thermal Inactivation Time at 41.5°C. of Herpes Simplex Virus, as Based upon the Length of Time Required to Destroy the Dermotropic and Neurotropic Factors of the Virus

Time virus heated at 41.5°C.	Results of corneal scarification and intracerebral inoculation of rabbits					
	Frank strain		H. F. strain		Go strain	
	Development of keratitis	Death from encephalitis	Development of keratitis	Death from encephalitis	Development of keratitis	Death from encephalitis
hrs.						
10	6th day	7th day	3rd day	5th day	2nd day	9th day
20	2nd "	6th "	5th "	6th "	2nd "	5th "
30	4th "	6th "	4th "	6th "	4th "	Remained well
40	5th "	7th "	4th "	5th "	4th "	" "
50*	No keratitis	6th "	No keratitis	6th "	No keratitis	" "
60	5th day	10th "	" "	13th "	" "	" "
70	No keratitis	8th "	" "	5th "	" "	" "
80†	" "	Remained well	" "	Remained well	" "	" "
90	" "	Died, intercurrent infection	" "	" "	" "	" "
100	" "	Remained well	" "	" "	" "	" "
Controls at 37°C.:						
0	3rd day	6th day	2nd day	7th day	2nd day	3rd day
100	4th "	7th "	4th "	8th "	2nd "	5th "

* Represents thermal inactivation time of Go strain.

† Represents thermal inactivation time of Frank and H. F. strains.

cause encephalitis until it had been heated for 80 hours. The Frank strain produced keratitis after exposure to 41.5°C. for 60 hours. After 70 hours of heating it no longer caused corneal involvement, but produced encephalitis. Animals injected with suspensions of the Frank strain, heated for 80, 90, and 100 hours, remained normal.

No marked difference was noted between the thermostability of the dermotropic and neurotropic factors of the recently recovered Go strain. Clinical signs of encephalitis and keratitis occurred simultaneously in rabbits inoculated with brain suspensions heated 30 and 40 hours, but the animals recovered from the infections. The rabbit injected with the suspension

exposed for 50 hours remained normal. Control rabbits injected with the 3 strains of virus which were kept for 100 hours at 37°C., developed keratitis and encephalitis, death occurring from 5 to 8 days after inoculation (Table I).

Histological examinations were made of the brain and of the corneal epithelium of all rabbits that died with symptoms typical of herpes encephalitis. Giemsa-stained sections revealed intranuclear inclusion bodies characteristic, morphologically and tinctorially, of those resulting from infection with herpes simplex virus.

DISCUSSION

Inasmuch as the nature of filtrable viruses is not well understood, the term "thermal inactivation time" is used in preference to "thermal death time," because of the implication of viability in the latter term. Whether the inability of the heated virus to produce keratitis or encephalitis was due to destruction of the virus, or merely to injury or alteration, is also unknown.

It is obvious that herpes virus is resistant to fever temperatures, and that the thermal inactivation time at such temperatures is longer than the duration of the fever which provokes its clinical manifestations in man.

It was anticipated, therefore, that a comparatively long exposure to a temperature of 41.5°C. would be required to inactivate the virus. If we assume that the virus is located in the superficial lesions of the skin, where the temperature is only 38° or 39°C. when the rectal temperature is 41.5°C., the thermal inactivation time *in vivo* would be much greater. The longest fevers given in this clinic, which were of 27 hours' duration at 41.5°C., were considerably shorter than the *in vitro* thermal inactivation time of the most susceptible strain of virus tested.

The recently recovered strain, Go, was less thermostable than the Frank and H. F. strains which were recovered from herpetic vesicles many years ago, and may be considered fixed by continual animal passage. It is possible that these two strains had already acquired some thermostability by prolonged exposure to a temperature of 38° or 39°C. in the rabbit.

SUMMARY

1. The thermal inactivation time at 41.5°C. of the H. F. and Frank strains of herpes simplex virus, under the conditions described, was 80 hours.

2. A strain of herpes virus recently recovered from a patient treated with a physically induced fever had a thermal inactivation time of 50 hours at 41.5°C.

3. The neurotropic factor of the H. F. and Frank strains of virus was

more resistant to a temperature of 41.5°C. than the dermotropic factor. There was little difference in the thermostability of these two factors of the Go strain.

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