Different Effects of Temperature on Type 1 and Type 2 *Herpesvirus hominis* in Cell Culture (36626)

J. O. OH AND N. SCHLENKE

Francis I. Proctor Foundation, University of California Medical Center, San Francisco, California 94122

There are 2 types of *Herpesvirus hominis* (HVH), each possessing distinct antigenic and biologic characteristcs (1). Type 1 HVH is recovered primarily from nongenital sites and is spread nongenitally. On the other hand, type 2 HVH ("genital herpesvirus") is recovered primarily from genital sites and is transmitted venereally or from mother to newborn infant.

It is known that temperature exerts a significant influence on herpesvirus infections both *in vitro* (2–9) and *in vivo* (10–12). Since there are many differences in biological activity between type 1 and type 2 HVH (1, 13), a study was conducted to compare the effects of temperature on types 1 and 2 HVH in cell culture. This report describes distinct thermal differences between type 1 and type 2 HVH in rabbit kidney cell cultures.

Materials and Methods. Eight strains of type 1 (VR_3, PH, Sheely, Tyler, W270-1, W270-4, W270-11, W270-12) and 7 strains of type 2 HVH (MS, Cornelius, Curtis, P124, P407, E91, X263) were tested. These strains include both recent isolates and laboratory strains with long histories of passage in various tissues (Table I). The sources of these viruses were described in a previous paper (13). All of the viruses were passed once or twice in primary cultures of rabbit kidney cells in our laboratory before use, and had titers ranging between 10^5 and 10^7 50% tissue culture infectious dose (TCD_{50})/ml. Primary cultures of rabbit kidney cells obtained from New Zealand white rabbits (1.5 kg body wt) were grown in tubes with medium 199, containing 20% heat-inactivated calf serum and fortified with 100 units of penicillin and 100 μg of streptomycin/ml. Two-week-old rabbit kidney cells were used in the study, and the number of cells in any given tube ranged between 8.0×10^4 and 1.6×10^5.

Results. In the first experiment, 8 strains of type 1 and 7 strains of type 2 HVH were used in a test to determine their ability to produce cytopathic effects (CPE) in rabbit kidney cells at various temperatures. Three sets of tube-cultures of these cells were inoculated with 1 ml amounts of serial 10-fold dilutions of each virus. The infected rabbit kidney cells were then incubated at 37° for 2 hr to allow for virus adsorption to the cells. At the end of the incubation period, each tube was washed twice with a total volume of 4 ml of phosphate buffered saline (PBS). Then 1 ml of fresh culture medium was added to each tube. Each set of kidney cell tubes was then reincubated at 30, 37, and 40°, respectively. CPE was recorded daily for 5 days, and the minimum infectious dose (MID) of the virus was determined. The MID was expressed as the highest dilution of the virus that produced CPE. The results of the experiment are shown in Table I.

The MID at 30 and 37° for all strains of both type 1 and type 2 HVH were not significantly different. As for the values at 40°, 3 out of 8 strains of type 1 HVH showed a somewhat lower MID than at 37°, and the remaining 5 strains showed no difference. On the other hand, all strains of type 2 HHV at this temperature produced only focal CPE with inocula containing as much as 10^{-1} dilution of the stock virus (equivalent to 10^4 or 10^5 TCD_{50}). The focal CPE observed in some tubes receded after a few additional days of incubation at 40° (Fig. 1). Normal kidney cells which had been incubated at 40°

TABLE I. Minimum Infectious Doses of *Herpesvirus hominis* for Rabbit Kidney Cells at 30, 37 or 40°.

Type	Strain: Name	Strain: Passage history[a]	Highest dilution of HVH producing CPE (log_{10}): 30°	37°	40°
	VR_3	LAB	−4	−5	−3
	PH	LAB	−6	−6	−3
	SHEELY	RI	−6	−5	−5
	TYLER	RI	−6	−6	−4
1	W270-1	UK	−5	−6	−2
	W270-4	UK	−5	−6	−3
	W270-11	UK	−4	−5	−4
	W270-12	UK	−4	−5	−4
	MS	LAB	−5	−5	−1[b]
	CORNELIUS	RI	−5	−5	−1[b]
	CURTIS	RI	−5	−5	−1[b]
2	P124	RI	−5	−5	−1[b]
	P407	RI	−5	−5	−1
	E91	RI	−4	−6	−1
	X263	RI	−4	−5	−1

[a] LAB, laboratory strain; RI, recent isolate; UK, unknown.
[b] Only focal CPE could be found.

for 5 days were fully susceptible to type 1 as well as type 2 HVH upon incubation at 37° following virus inoculation.

In the second experiment, 4 strains each of type 1 (VR_3, PH, Sheely, Tyler) and type 2 HVH (MS, Cornelius, Curtis, P124) were used to study the multiplication of the virus at 30, 37, and 40°. Three sets of duplicate rabbit kidney cell tubes were infected with approximately 10^3 TCD_{50} of HVH and incubated at 37° for 2 hr. As in the case of the preceding experiment, the infected kidney cells were then washed twice with PBS, and each tube received 1 ml of fresh culture medium. Each of the 3 sets of kidney tubes was then reincubated at 30, 37 and 40°, respectively, for 2 days. At the end of the incubation period, the kidney cells were subjected to 3 cycles of rapid freezing and thawing and were then centrifuged at 800*g* for 10 min at 4°. The infectivity of the pooled supernatant from the duplicate kidney tubes of each set was titrated in rabbit kidney cell tubes. As shown in Table II, both type 1 and type 2 HVH grew slower at both 30 and 40° than at 37°, but this growth differential was even more pronounced in cultures infected with type 2 strains of HVH, especially at 40°.

In the final experiment the nature of the abortive infection of type 2 HVH was examined. Three strains of type 2 HVH (MS, Curtis, Cornelius) were used to study the production of CPE in kidney cells infected with virus which had been maintained at 40° followed by a transfer to 37°, or vice versa. Five sets of kidney cell tubes were inoculated with 1 ml of serial 10-fold dilutions of each virus and incubated at 37° for 2 hr. Then each tube was washed with PBS and received 1 ml of fresh cell culture medium as outlined in the first experiment. One set was maintained at 37° throughout the entire period of 12 days. Four sets of the kidney tubes were incubated at 40°. On postinfection days 1, 3 and 6, respectively, one set each was transferred to 37°, and a remaining set was maintained at 40° until the end of the experiment. CPE was recorded daily for 12 days, and the daily MID of the virus was determined. As shown in Fig. 1, suppression of CPE was observed in the cells inoculated

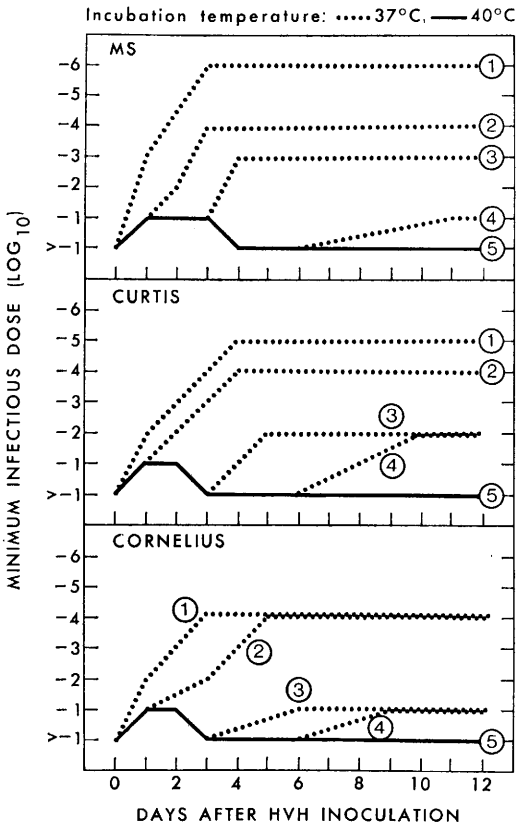

FIG. 1. Minimum infectious doses of type 2 *Herpesvirus hominis* following incubation at 40°, then at 37°. The minimum infectious dose is the highest dilution of the virus that produces cytopathic effects. (1) incubated at 37° for the entire period; (2) at 40° for 1 day, then at 37° thereafter; (3) at 40° for 3 days, then at 37° thereafter; (4) at 40° for 6 days, then at 37° thereafter; (5) at 40° for the entire period.

with most dilutions of the virus as long as they were maintained at 40° (line 5). However, upon transferring the cells to 37°, CPE started to appear. If the infected cells were transferred to 37° after incubation at 40° for 1 day, CPE was observed in the tubes inoculated with low as well as high concentrations of the virus. When the duration of incubation at 40° was prolonged prior to transfer to 37° (3 or 6 days), the CPE could be observed only in the tubes which had received high concentrations of the virus. The appearance of CPE in the cells following transfer to 37° was accompanied with rising infectivity titers in the cells as shown in Table III.

A similar experiment was carried out to study the production of CPE in kidney cells by type 2 HVH which had been incubated at 37° for 1 day followed by incubation at 40°. As shown in Fig. 2, further spread of CPE produced by type 2 HVH following 1 day of incubation at 37° was successfully blocked by incubating the infected cells at 40°.

Discussion. Recently Ratcliff (9) reported that all the type 1 strains of HVH he tested produced CPE in monolayers of Vero cells at 35, 39.2, 39.8 and 40.3°, but the production of CPE by type 2 strains was markedly inhibited at temperatures above 39°. Our data in the present experiment are in complete agreement with his findings. Furthermore, our study revealed that the inhibition of CPE production by type 2 HVH at 40° was accompanied by a marked reduction of virus multiplication in the cells. Our results as well as the results of others (8, 9) have strengthened the concept that a temperature-marker test might be used as a simple alternative test for the differentiation of type 1 and type 2 strains of HVH.

Another interesting finding in our study was that the inhibition of both CPE production and virus synthesis of type 2 HVH strains at 40° could be converted to active CPE production and virus growth by transferring the infected cells to 37°. Crouch and Rapp (7) recently reported that in rabbit

TABLE II. Growth of *Herpesvirus hominis* in Rabbit Kidney Cells at 30, 37 or 40°.

Type	Strain	Titer following 2-day incubation[a] (log_{10} TCD_{50})		
		30°	37°	40°
1	VR_3	6.1	6.5	5.0
	PH	4.1	5.0	4.5
	Sheely	5.1	6.0	5.4
	Tyler	5.2	7.1	6.5
2	MS	3.1	5.0	2.5
	Cornelius	2.0	4.1	1.8
	Curtis	3.0	5.0	1.1
	P124	4.1	5.3	1.1

[a] Initial inoculum: 10^3 TCD_{50}/tube of kidney cells.

TABLE III. Growth of Type 2 *Herpesvirus hominis* in Rabbit Kidney Cells Incubated at 40° Then at 37°.[a]

Virus strain	Virus titer (TCD_{50}/ml)			
	Inoculum	After 3-days incubation at 40°	After additional 1-day incubation at 37°	After additional 1-day incubation at 40°
MS	$10^{3.0}$	$<10^{1.0}$	$10^{4.2}$	$10^{1.1}$
Curtis	$10^{3.1}$	$10^{2.0}$	$10^{5.4}$	$<10^{1.0}$
Cornelius	$10^{3.3}$	$<10^{1.0}$	$10^{2.8}$	$10^{1.1}$

[a] Approximately 10^3 TCD_{50} of a strain of type 2 HVH were inoculated into each of 12 rabbit kidney cell tubes and incubated at 37° for 2 hr. The cells were then washed twice with a total of 4 ml of PBS, and incubated with 1 ml of fresh culture medium at 40° for 3 days. At the end of the incubation period, 4 tubes were further incubated for 1 day at 37° and another set of 4 tubes at 40° prior to virus titration. The remaining 4 tubes were used for virus titration without additional incubation. For infectivity titration, kidney cells in 4 tubes were subjected to 2 cycles of rapid freezing and thawing and centrifuged at 800*g* for 10 min. The pooled supernatant from 4 tubes was used for virus assay.

and hamster cells infected with HVH (strain 307), the amount of viral DNA synthesized at 39° was greatly reduced in both cell systems. This may account for the suppression of both CPE production and virus growth in rabbit kidney cells infected by type 2 strains of HVH at 40° as observed in our study. However, the exact mechanisms by which high temperatures produced the observed reversible abortive infections with type 2 HVH are not clear at present. A similar reactivation of an abortive infection was observed in KB cells infected with type 2 adenovirus (14). In KB cells, the production of infectious virus was reduced more than 99% when infected cell suspension cultures were maintained at 42°, while cultures held at 42° for 12 hr and then shifted to 37° yielded normal amounts of infectious virus. The authors attributed the failure of virus synthesis at the elevated temperature to lack of capsomere assembly. Further studies are needed to test whether similar mechanisms may be operative in the abortive infection of type 2 HVH at 40°.

Our finding that type 2 HVH produces reversible abortive infection in rabbit kidney cells at elevated temperatures may have clinical importance. Type 2 HVH is associated mainly with the genital tract and produces cervical, vaginal, or urethral infections. In view of the fact that the temperature of genital tract (endocervix) is around 37° for normal women (15) and it will be elevated above 37° in the person with genital tract infections, the potential conditions for an abortive infection of type 2 HVH, such as we have observed in the rabbit cells, exist in such patients. It is quite possible that a higher temperature in the target organ, such as the cervix, may play an important role in the pathogenesis of type 2 HVH infection and, possibly, in the oncogenic capabilities of the virus. Studies of such possibilities *in vivo* would seem to be warranted although certain characteristics of an *in vitro* viral infection may not necessarily contribute to our understanding of the pathogenesis of lesions produced *in vivo*.

Summary. Effects of various incubation temperatures (30, 37 and 40°) on type 1 and type 2 strains of *Herpesvirus hominis* (HVH) were studied in primary cultures of rabbit kidney cells. Eight strains of type 1 and 7 strains of type 2 HVH were used. These strains included both recent isolates and laboratory strains with long histories of passage in various tissues.

Both type 1 and type 2 HVH strains, regardless of passage history, grew slower at 30 and 40° than at 37°, but this retardation of growth was more pronounced in cultures in-

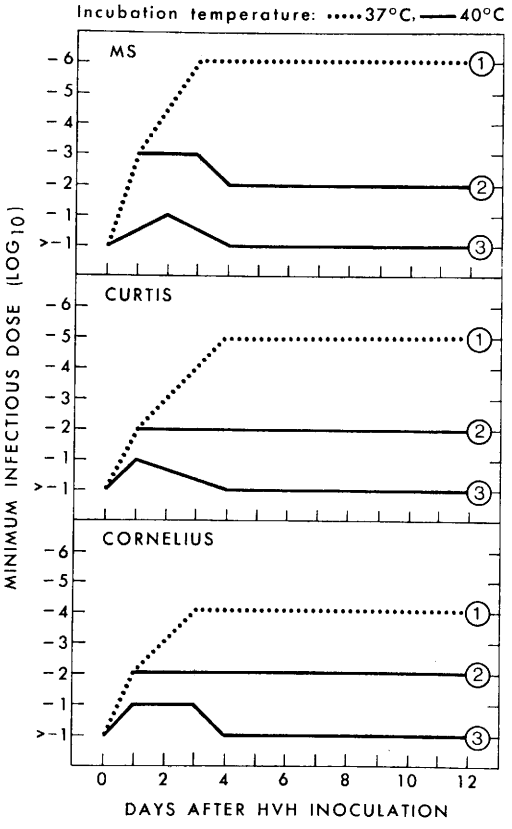

FIG. 2. Minimum infectious doses of type 2 *Herpesvirus hominis* following incubation at 37°, then at 40°. (1) at 37° for the entire period; (2) at 37° for 1 day, then at 40° thereafter; (3) at 40° for the entire period. The minimum infectious dose is defined in Fig. 1.

fected with type 2 strains of HVH at 40°. The production of cytopathic effects (CPE) and the growth of type 2 HVH in rabbit kidney cells were almost completely suppressed as long as the infected cells were maintained at 40°. Upon transferring the cells to 37°, however, CPE along with rising infectivity titers of the virus were noted. Further spread of CPE produced by type 2 HVH following incubation at 37° was successfully blocked by incubating the cells at 40°.

This work was supported by Grant EY 00310 from the National Eye Institute.

1. Nahmias, A. J., and Dowdle, W. R., Progr. Med Virol. **10**, 110 (1968).

2. Thompson, R. L., and Coates, M. D., J. Infec. Dis. **71**, 83 (1942).

3. Farnham, A. E., and Newton, A. A., Virology **7**, 449 (1959).

4. Hoggan, M. D., and Roizman, B., Virology **8**, 508 (1959).

5. Wheeler, C. E., and Canby, C. M., J. Immunol. **83**, 392 (1959).

6. Yoshino, K., Taniguchi, S., and Takeuchi, H., Jap. J. Microbiol. **12**, 97 (1968).

7. Crouch, N. A., and Rapp, F., J. Virol. **9**, 223 (1972).

8. Longson, M., Ann. Inst. Pasteur, Paris **120**, 699 (1971).

9. Ratcliff, H. J., J. Gen. Virol. **13**, 181 (1971).

10. Schmidt, J. R., and Rasmussen, A. F., J. Infec. Dis. **107**, 356 (1960).

11. Lycke, E., Hermodsson, S., Kristensson, K., and Roos, B. E., Acta Pathol. Microbiol. Scand. **79**, 502 (1971).

12. Warren, S. L., Carpenter, C. M., and Boak, R. A., J. Exp. Med. **71**, 155 (1940).

13. Oh, J. O., Moschini, G. B., Okumoto, M., and Stevens, T., Infec. Immunity **5**, 412 (1972).

14. Okubo, C. K., and Raskas, H. J., Virology **46**, 175 (1971).

15. Arronet, G. H., Fert. Steril. **8**, 301 (1957).

Received Apr. 11, 1972. P.S.E.B.M., 1972, Vol. 140.