COMPARATIVE STUDIES OF TYPE 1 AND TYPE 2 "HERPES SIMPLEX" VIRUSES*

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THE various strains of the so-called "herpes simplex virus" fall into 2 distinct antigenic groups referred to as Type 1 and Type 2 (Plummer, 1964; Pauls and Dowdle, 1967). Recent findings clearly demonstrate that the type 1 strains cause infections of the mouth whereas the type 2 strains are responsible for herpes genitalis (Dowdle, Nahmias, Harwell and Pauls, 1967).

Until this work demonstrated that genital lesions were a source of type 2 strains, only the MS and US strains of this type were available for study. The former was isolated from the central nervous system of a case of multiple sclerosis; the latter is of unknown origin. Extensive studies of these 2 isolates have indicated a number of differences between them and the ordinary type 1 isolates from the mouth, though the significance of the observations was somewhat uncertain because only 2 type 2 isolates were available. Differences were noted in the cytopathic effect produced in tissue culture and the much lower levels of infective virus attained by the MS and US strains in tissue cultures; in other words the type 2 strains showed much less "efficiency" than type 1 strains in tissue culture. Somewhat in contrast to this the type 2 isolates were markedly more neurotropic in laboratory animals (Plummer and Hackett, 1966; Plummer, Cleveland and Stevens, 1967). It is of interest that Lehel and Hadhazy (1966) in their study of the inhibitory effects of heparin on 6 strains of simplex virus in the skin of rabbits noted that their 1 strain which was of genital origin was not inhibited as much by heparin as the other strains, and that it was also more neurovirulent than the other strains and that its cytopathic effect in tissue culture was different.

The ready availability of further type 2 strains from herpes genitalis has made possible the extension of the comparative study and confirmation of the earlier observations concerning neurovirulence and other biological features. This paper presents the results of the work.

MATERIALS AND METHODS

Viruses—Type 1 strains: L2, isolated in Russia by Shubladze, Maevskaya, Ananev and Volkova (1960) from the mouth, but is of unknown passage level. Strain BW, isolated from the mouth and passed at least 20 times in rabbit kidney cultures. Strain Watson was isolated from the lip, and strains 197 and 356 from the pharynx, and each has been passed 5 times in rabbit kidney cultures. Type 2 strains: MS was isolated by Dr. M. Gudnadottir from the CNS of a case of multiple sclerosis and has been passed 14 times in rabbit kidney cultures. The US strain was isolated by Shubladze *et al.* (1960) but is of unknown

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origin or passage level. Strains Wiggins and Dawson were obtained from the cervix and passed 5 times in rabbit kidney cells. D64 came from a generalized infection of an infant and has been passed 7 times in tissue cultures. (Strains Watson, 197, 356, Wiggins, Dawson and D64 were obtained through the courtesy of Drs. W. R. Dowdle and A. J. Nahmias.) The various stocks of viruses used in the experiments were grown in rabbit kidney cultures.

Growth curves.—All tissue cultures were maintained on 199 with 5 per cent lamb serum. A growth curve was not done in a single tissue culture, as this would have made it impossible to determine the levels of intracellular virus. Young confluent rabbit kidney cultures in tubes were inoculated in parallel, each receiving 2·0 $\log_{10}$ PFU per tube, with 20 min. absorption onto the drained cell sheet, after which it was washed with one change of medium prior to the replacement of the maintenance medium. At the intervals at which the titrations were done, the culture fluid was removed and titrated, and the infected cell sheet was washed and then scraped off with a rubber policeman into 1 ml. of medium, the mixture then being homogenized in a Ten-Broek grinder; the fine debris was centrifuged out and the supernatant titrated. Titrations were done in petri-dish cultures of rabbit kidney cells with a methocel/199 overlay.

Inactivation curves.—Infected cultures in 16 oz. prescription bottles were used. The stability of the extracellular virus from the culture fluid was determined by passing through a 3 μ millipore filter and diluting the filtrate 1/10 in 199 containing antibiotics and 0·1 per cent sodium bicarbonate but with no serum. The diluted material was held in a tightly stoppered 4 oz. prescription bottle at 37° from which samples for titration were taken at intervals. The stability of the cell associated virus was determined in a similar manner after extracting it from the cell sheet. After thrice washing the sheet the cells were scraped off with a rubber policeman into 3 ml. of medium and were homogenized in a Ten-Broek grinder. The homogenized material was passed through a 3 μ filter and the filtrate diluted 1/10. This diluted material was also stored in a stoppered bottle and titrated at intervals.

Heparin tests.—The effect of heparin (Panheprin from the Abbott laboratories) on the 2 types was determined in rabbit kidney cultures. Each virus strain was inoculated at a concentration of 1000 PFU per tube culture 1 hr. after the addition of heparin to the culture fluid at a concentration of 100 USP units/ml. of fluid. The cultures were observed for several days and the rate of development of CPE was recorded and compared with the rate of progress of CPE in cultures receiving similar inocula of virus but no heparin.

RESULTS

Behaviour in tissue cultures

The CPE in both rabbit kidney and human lung fibroblast cultures produced by the 5 type 2 strains consistently differed from that produced by the 5 type 1 strains, making it possible to identify the type to which an isolate belonged on the basis of CPE alone. All the cells involved in the CPE of the type 2 viruses became swollen and rounded, taking on a smooth globular appearance. The appearance of such a focus of CPE was thus very similar to that of human cytomegalovirus in human fibroblast cultures. In contrast, the cells involved in the CPE of type 1 strains did not all take on a similar appearance; some of them did have the swollen rounded shape seen with type 2, but most of them did not become completely separated from their neighbouring cells and hence retained some of their original shape. Foci of CPE produced by type 1 virus were thus less easy to see when viewed under the low power of the microscope. These differences were particularly marked when plaques of CPE under a solid overlay were examined.

The titres of infective virus attained in the culture fluid by type 2 viruses were much lower than those attained by type 1 strains. In a series of experiments in which tissue cultures were infected with similar doses of different strains of the 2 types, the maximum titres of infective virus reached in the culture fluid were about 100–1000-fold lower for type 2 than for type 1. This is illustrated

by the growth curves presented in Fig. 1, from which it can also be seen that the intracellular virus levels are also lower for type 2 than for type 1. The reasons for this are not entirely clear, though the inactivation curves shown in Fig. 2 demonstrate that both intra- and extracellular type 2 virus are less stable in tissue culture fluid than type 1 virus, which may, in part at least, account for the differences in amounts of infective virus. Inactivation curves were also

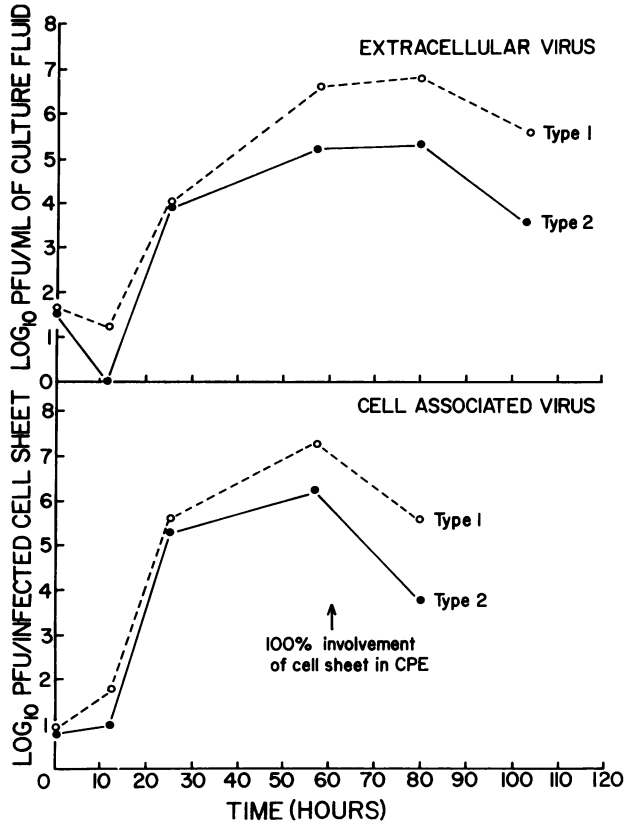

FIG. 1.—Comparative growth curves of type 1 (strain 197) and type 2 (strain D64) viruses in rabbit kidney tissue culture.

obtained for strains of type 1 and type 2 other than those in Fig. 2; they also showed the greater instability of the type 2 strains.

Sensitivity of the viruses to heparin

There has been some indication that the type 2 strains are less sensitive to the inhibitory action of heparin than are the type 1 viruses (Lehel and Hadhazy, 1966). All the strains of type 1 and of type 2 were therefore compared in this respect. The type 2 strains were in fact less inhibited than the type 1 strains. This is shown by the curves in Fig. 3, which represent the rates of development of CPE in control cultures and in cultures containing 100 units of heparin per ml.

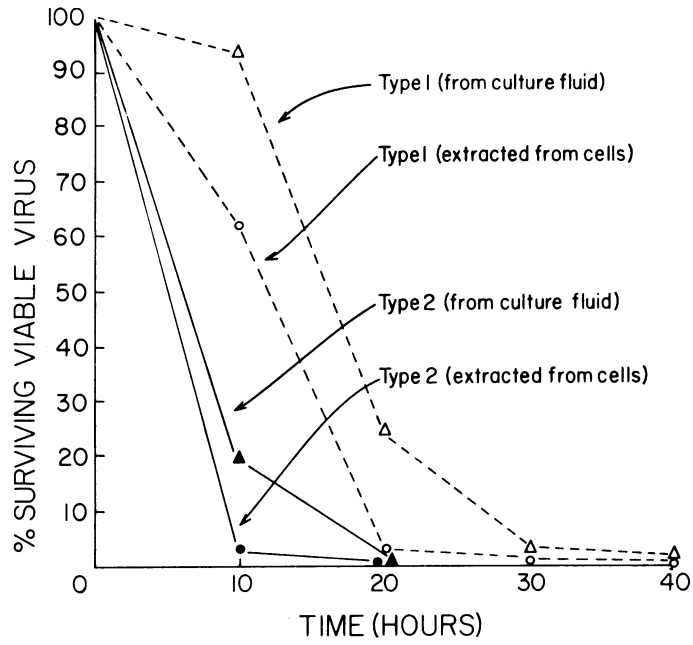

FIG. 2.—Inactivation curves of type 1 (strain 197) and type 2 (strain MS) viruses at 37° in medium 199.

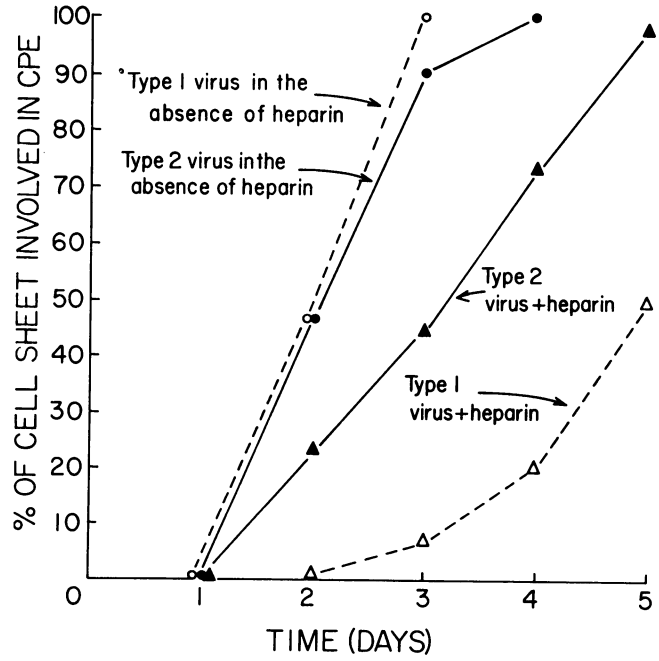

FIG. 3.—The inhibitory effect of heparin on type 1 (strain L2) and type 2 (strain MS) viruses.

Neurotropic properties

The ability to invade the CNS of laboratory animals when introduced into muscle was determined by the inoculation into the femoral muscle of the left back leg of 5-month-old New Zealand rabbits of 10^4 PFU of virus, and into the corresponding muscle of one-month-old Swiss-Webster mice of 10^3 PFU of virus. The animals were observed for the development of paralysis or myelitis. As can be seen from Tables I and II, the type 2 strains are much more neurotropic

TABLE I.—*Neurotropic Properties in Adult Rabbits of Type* 1 *and Type* 2 *Strains of " Herpes Simplex Viruses " Inoculated i.m. into a Back leg*

Type 1			Type 2		
Strain	Titre of inoculum (PFU)	Numbers developing paralysis	Strain	Titre of inoculum (PFU)	Numbers developing paralysis
Watson	10^4	0/5	Wiggins	10^4	6/11
Watson	10^6	0/6	D64	10^4	3/10
197	10^4	0/5	Dawson	10^4	3/10
197	10^6	0/6	US	10^4	7/10
356	10^4	0/5	MS	10^4	6/10
L2	10^6	0/10			
BW	10^6	0/11			

TABLE II.—*Neurotropic Properties of Type* 1 *and Type* 2 *Strains in Mice, Indicated as Numbers of Inoculated Mice Showing CNS Involvement*

Type 1		Type 2	
197	0/36	Wiggins	13/35
Watson	3/34	Dawson	16/33
L2	1/22	MS	12/20

than the type 1 strains. To test further the lower neurovirulence of the type 1 strains, groups of 5-month-old rabbits were inoculated with the type 1 strains using 100-fold more virus than in the previous experiment (*i.e.* the inoculum was 10^6 PFU per rabbit). None of these animals developed clinical involvement of the CNS.

Histological examination of 2 of the rabbits, 1 paralyzed by the Wiggins strain and the other by the Dawson strain, showed inflammation of the appropriate dorsal ganglia and horns similar to that previously shown (Plummer *et al.*, 1967) for strains MS and US.

DISCUSSION

The type 2 strains of " herpes simplex " clearly differ from the type 1 strains in the following respects: (1) They are antigenically different when compared in the cross neutralization tests. (2) Their behaviour in tissue cultures is different in regard to cytopathic effect and the amounts of viable virus detectable in the infected cultures, which may be partly or completely explicable by the greater

instability of the type 2 strains. (3) Type 1 strains are responsible for mouth infections of humans and the type 2 strains for genital infections. (4) The type 2 strains are considerably more neurotropic in laboratory animals than are the type 1 strains.

The greater neurotropic activity of the type 2 strains in mice and rabbits is quite clear from the comparative results presented in this paper. In these species of animals, however, the age of the animal and the titre of the virus inoculated do to some extent influence the outcome. Thus in earlier studies in which type 1 virus of a much higher titre was used as an inoculum for mice, there was neurotropic activity, though even then it was less than with the type 2 strain with which it was compared (Plummer and Hackett, 1966); neurotropic activity could also be demonstrated in very young rabbits into which large quantities of a type 1 strain were inoculated (Plummer *et al.*, 1967).

The isolates studied by Dowdle *et al.* (1967) from herpes encephalitides of humans were of type 1. This raises the question of the neurotropic activity of the type 2 strains in humans, especially in the light of their greater neurotropic activity in laboratory animals and the origin of the MS strain from the CNS of a case of multiple sclerosis. A variety of studies by other workers of herpes simplex virus in small laboratory animals have indicated that invasion of the central nervous system is via a nervous pathway so that, bearing in mind the genital distribution of the type 2 lesions in humans, it would be expected that the type 2 virus would invade the lower spine rather than the brain. The subsequent rapid invasion of other parts of the central nervous system would not necessarily be expected in view of the frequent examples of non-fatal herpetic encephalitides (*e.g.* Gostling, 1967) and examples of difficulty of isolating virus from such encephalitides, even to the extent of not being able to isolate virus from several regions of the brain when it was readily obtainable from one region (*e.g.* Grist, 1967). Such cases indicate that simplex virus does not necessarily rapidly extend itself either clinically or multiplication-wise to all parts of the CNS even though it may be already established and active at one point.

The possible involvement of herpesviruses in slow diseases of the CNS needs more study. It is perhaps of interest that all of 9 sera examined from patients with amyotrophic lateral sclerosis had neutralizing antibody levels to herpes simplex virus that were 4- to 8-fold higher than that in any of 50 control sera of a similar age group (Plummer, unpublished). A greater frequency of complement fixing antibodies to varicella/zoster virus was noted by Ross, Lenman, and Rutter (1965) among sera from multiple sclerosis patients than among control sera. These observations may, however, have no significance in regard to the aetiology of these diseases; the incidental activation of herpesviruses already latent in the CNS by the degenerative processes of the diseases concerned could well account for the boosting of antibody levels to them.

SUMMARY

Five strains of herpes simplex virus type 1 (all isolated from the mouth) and five of type 2 (3 from herpes genitalis, one from the spine of a case of multiple sclerosis, and one of unknown origin) were compared. It was concluded that in addition to their antigenic difference and their clinical difference in humans (*i.e.* type 1 causing mouth infections and type 2 genital infections) they also

differed in their behaviour in rabbit kidney tissue culture in respect of cytopathic effect, and lower levels of infectivity attained by, and greater instability shown by, the type 2 virus. The type 2 strains were also markedly more neurotropic in rabbits and mice than the type 1 strains.

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