JOURNAL OF VIROLOGY, Jan. 1969, p. 95-97

Vol. 3, No. 1
Printed in U.S.A.

Oral and Genital Bovine Herpesviruses

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Received for publication 1 October 1968

Genital strains of herpes simplex virus are serologically different from the mouth and eye strains (1, 8, 9, 11). The genital form is called herpes simplex virus type 2, and the serotype from the mouth and eyes, type 1. The two viruses differ in several other ways including cytopathic effect in tissue culture (2, 10), greater instability of the type 2 virus when grown in tissue cultures, and the lower titers obtained by it in these cultures (2, 10). The densities of the deoxyribonucleic acid (DNA) of the two viruses are different, indicating a 2% difference in their guanine plus cytosine (GC) ratios (4). Interest in the type 2 virus has recently increased because of suggestions (12) that it is the cause of cervical cancer.

There is a genital herpesvirus infection of cattle that causes vulvovaginitis and lesions of the penis that are very similar to herpes genitalis of man (5, 6, 14, 15). It has been assumed that the virus concerned is the common respiratory virus of cattle, infectious bovine rhinotracheitis virus, although the respiratory and genital symptoms were not necessarily together in the same herd. Thus, herpes genitalis of cattle does not usually accompany an outbreak of rhinotracheitis, and herpes genitalis can be present venereally in a herd without respiratory symptoms. The comparative studies that have been carried out (3, 7) of the respiratory and genital bovine viruses have indicated that by the techniques which were used the two are similar if not one and the same virus. In view of the more recent work with the human viruses, we reexamined the relationship between the genital and respiratory bovine herpesviruses using exactly the same techniques that have differentiated herpes simplex type 2 from type 1.

The following strains were compared serologically: two respiratory strains, LA and Col; two genital strains, K and NY; one isolated from the genital tract of an animal which also had respiratory disease, strain Mont; one isolated from bovine keratoconjunctivitis, strain PE; and one isolated from an aborted bovine fetus, strain B. Strains LA, K, and B were obtained from D. G. McKercher, strain NY was obtained from J. H. Gillespie, and strain PE was obtained from Leon Dmochowski. Specific antisera were prepared in rabbits against strains LA, Mont, NY, and B. The rabbits were inoculated with culture fluid from infected rabbit kidney cultures, 3 ml intramuscularly into a hind leg and 3 ml intraperitoneally; similar inoculations were given 3 weeks later, and the animals were bled 1 week later. Neutralization curves at 37 C were performed between each serum and each of the six virus strains. The sera were diluted 1:20, prewarmed, and mixed with a prewarmed virus suspension, previously diluted so that there would be 750 plaque-forming units/0.2 ml of the final mixture. A similar control mixture was made of virus and preimmunization rabbit serum prediluted 1:20. The control was titrated immediately and was regarded as the 0-min sample; the test sample was titrated at 5, 20, and 40 min, and the control mixture was titrated once more at 40 min. All titrations were carried out by the plaque technique using rabbit kidney cultures and an overlay of 199/lamb serum hardened by methocel. No distinction could be made among any of the 6 strains by the 24 neutralization curves. Figures 1A and 1B show LA antiserum against LA virus and NY virus, and NY antiserum against NY virus and LA virus. In contrast, Fig. 1C and 1D show type 1 and type 2 herpes simplex antisera against type 1 and type 2 herpes simplex virus; as can be observed from the latter figures, the serological difference between the oral and genital human herpesviruses is considerable.

The densities of the DNA of two respiratory strains (LA and Col) and two genital strains (K and NY) were measured to determine if they differed in a way similar to the human genital and oral viruses (4). Each virus was grown in primary rabbit kidney cultures, and after the development of advanced cytopathic effect, the DNA was extracted from the cells by the method of Russell and Crawford (13). The mixture of viral and cellular DNA was centrifuged to equilibrium in cesium chloride, and scanner tracings were made. All centrifugation runs were for 24 to 48 hr at 44,000 rev/min, 25 C, initial density 1.70 g/ml, in a double-sector, aluminum-filled Epon cell of the Spinco Model E analytical ultracentrifuge

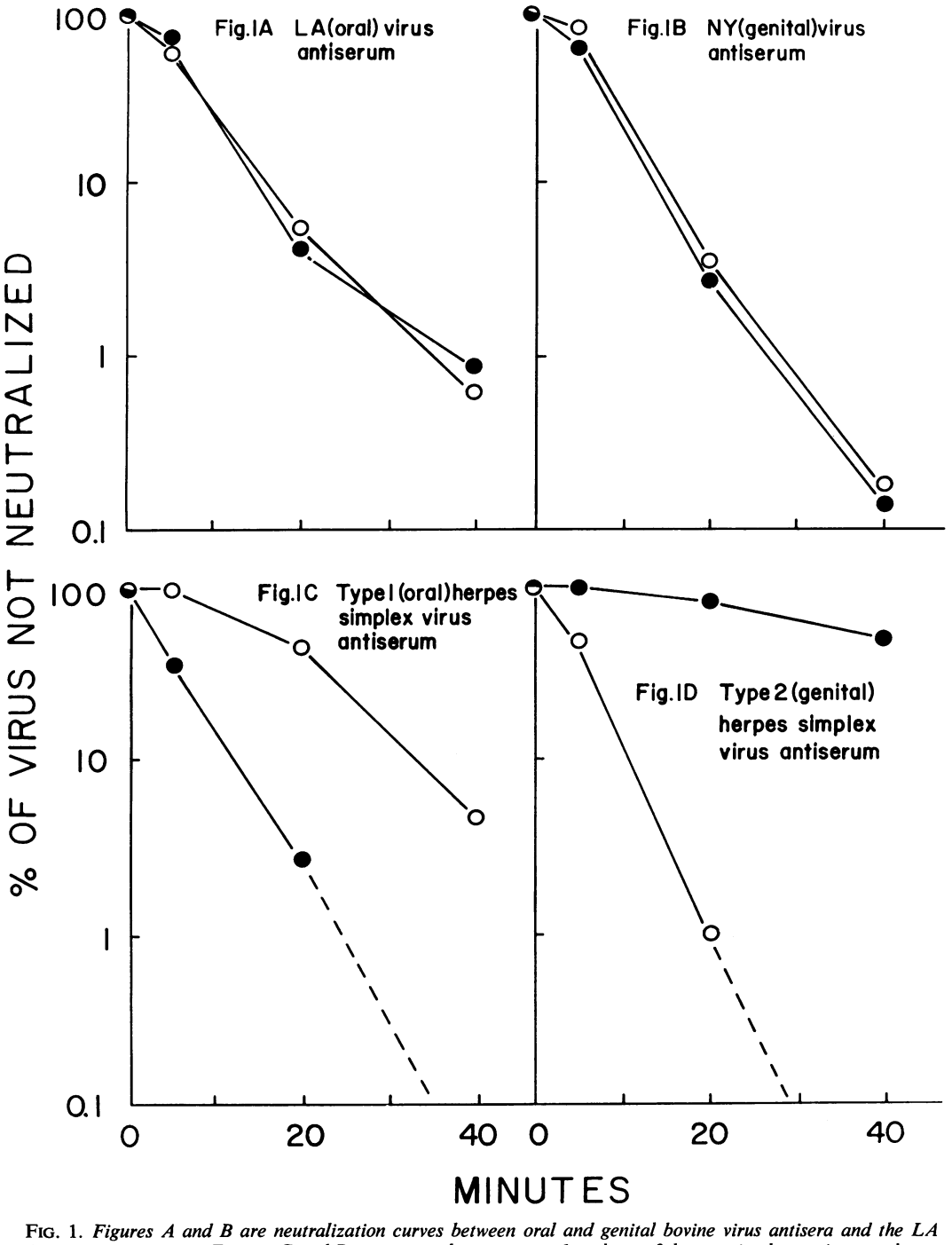

FIG. 1. *Figures A and B are neutralization curves between oral and genital bovine virus antisera and the LA and NY strains of virus. Figures C and D are curves between type 1 and type 2 herpes simplex antisera and type 1 and type 2 viruses. The control curves, using preimmunization sera, are not shown; in none of them was there a drop in virus titer of more than 10% during the 40-min incubation. Oral virus, ●; genital virus, ○.*

equipped with monochromator, ultraviolet absorption optics, and electronic scanner. Two distinct peaks, one viral and one cellular, were obtained with each virus-cell mixture. The density of the cell peak, determined in a separate run by mixing rabbit DNA with *Clostridium perfringens* DNA (1.691 g/ml), was 1.699 g/ml. The positions of peaks were determined with the DuPont Curve Resolver (model 310). In this way, the error of locating even such a broad peak as occurs with mammalian DNA corresponds to density differences of less than ± 0.0008 g/ml. The positions of all four viral peaks were the same, indicating a density of 1.731 g/ml for each of them. The results suggest that the GC contents of the two genital strains were the same as those of the two respiratory strains, i.e., 72%.

The cytopathic effects produced by all six strains of virus in rabbit kidney cultures were similar, as were the levels of viable virus attained per ml of culture fluid in cell cultures receiving similar inocula of virus. Once again, no differences were observed between the genital and oral isolates, leading to the conclusions that although they may differ in their natural bovine host, no dichotomy parallel to the human herpesviruses types 1 and 2 could be detected by the serological and density studies which we performed.

This investigation was supported by National Science Foundation research grant GB 6515.

The technical assistance of Janet Eby is gratefully acknowledged.

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