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RECOVERY OF HERPES SIMIAE (B VIRUS) FROM BOTH PRIMARY AND LATENT INFECTIONS IN RHESUS MONKEYS

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Summary.—The suspected ability of herpes simiae (B virus) to persist in a latent form has been confirmed in rhesus monkeys. The virus was recovered from primary oral lesions of 2 young monkeys and again, 6 months after disappearance of symptoms, from cultures of Gasserian ganglia taken from the same individuals. B virus was identified by its effects *in vivo* and *in vitro* and in cross neutralization tests with antisera to reference B virus and herpes simplex virus. Tests showed that the same virus was present in oral lesions and in ganglia. The one-way immunological relationship between herpes simplex virus and B virus was clearly shown in results of cross neutralization tests.

NUMEROUS antibody assays have suggested that herpes virus simiae (B virus) infection may be fairly wide-spread in Old World monkeys, particularly after they have been held captive for a time in simian colonies. The possibility has been raised that latency occurs in monkeys as it does in humans infected with the closely related herpes simplex virus (HSV) who may also show recurrent symptoms such as "cold sores" when stressed. There is no clear evidence that recrudescence of oral sores in monkeys occurs following the primary infection since such animals are usually slaughtered for safety. Nevertheless, B virus has appeared in cultures from tissues of "normal" monkeys and humans bitten by "normal" monkeys have died.

Sensory ganglia have been shown to harbour latent HSV: Stevens and Cook (1971) report the isolation of this virus from spinal ganglia of experimentally infected mice for up to 3 months after recovery from paralysis following HSV inoculation into the footpad. Stevens, Nesburn and Cook (1972) recovered HSV from trigeminal ganglia of rabbits 6 months after experimental infections. Simian herpes virus (Herpes tamarinus and the related KM 322) has recently been recovered from fragment cultures of the dorsal root ganglia of all experimentally infected rabbits killed up to 28 months after intradermal inoculation and, furthermore, free infectious virus was recovered from reactivated skin lesions which appeared when carriers were given cortisone treatment (McCarthy and Tosolini, 1975).

This work describes the recovery of B virus from the primary oral lesions of young rhesus monkeys and again, 6 months after the disappearance of symptoms, from explant cultures of the Gasserian ganglia of the same animals. A preliminary report of these findings has been published (Vizoso, 1975*a*).

MATERIALS AND METHODS

An outbreak of B virus infection occurred in a colony of rhesus monkeys in quarantine soon after their arrival from India. Three young animals were selected, all showing a similar stage of infection, which suggested that each might have contracted the disease at the same time and from the same source.

Swabs of oral herpetic lesions were taken

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from each animal, washed in tissue culture medium and the supernatants inoculated intracerebrally into suckling hamsters and suckling mice (6 litters per swab), subcutaneously into young albino rabbits (3 animals per swab) and into tissue cultures of VERO cells. One monkey died and the 2 survivors (Rh1 and Rh3) were kept without risk of infection from human or subhuman primates and in separate cages for 6 months. They were then anaesthetized with Nembutal (pentobarbitone sodium), killed by exsanguination and their Gasserian ganglia removed aseptically. A sample of serum was also taken. The ganglia were minced into fragments (0·5–1·0 mm^3), washed 3 times in phosphate buffered saline and the pieces cultured in plastic Falcon Flasks (5 per ganglion) containing Glasgow-modified Eagle's Minimum Essential Medium and 10% foetal calf serum. No attempt was made to fix the explants to the bottom of the flask. The procedure is similar to that described by Stevens and Cook (1971).

Samples of the supernatants from the ganglion cultures were tested for the presence of B virus by inoculation into VERO cell cultures and subcutaneous injection into young albino rabbits. They were also titrated by intracerebral inoculation in suckling hamsters and suckling mice. The cultures were kept and when the cells had formed confluent monolayers they were trypsinized and subcultured. Supernatants were tested several times at each passage for the presence of B virus by inoculation into VERO cell cultures. At the third passage the ganglion cell cultures were challenged by inoculation of the B virus originally recovered from the explant cultures. A total of 10 passages was made.

Serum neutralization tests on samples taken from the monkeys at the time of original infection and 5 months later were made in VERO cell cultures. Doubling dilutions of serum in plastic plates (LINBRO 15-FB-96) were mixed with 100 TCD_{50} of virus, one well per dilution, and kept at 37° for 1–2 h before the cells were added. Each well received 0·05 ml serum dilution, 0·05 ml virus dilution and 0·1 ml of a suspension containing 1–2 $\times$ 10^4 cells. Tests were read on the fourth day.

Viruses used in these tests were: B virus Cyno 2 isolated from the tongue of a cynomolgus monkey and identified by neutralization with specific antiserum (Glaxo Laboratories); B virus Rh1 and B virus Rh3 each isolated from one of the rhesus monkeys at the time when oral lesions were apparent and again from ganglion explants derived from the same animals when they were killed 6 months later; Herpes simplex T1 given by the late Professor F. Fulton, and SA8 strain 264 isolated from a vervet monkey and kindly supplied by Dr H. Malherbe. SA8 was used in neutralization tests because it produces a CPE in tissue cultures of VERO cells similar to that induced by B virus and the possibility of its presence needed to be excluded.

Antisera used were B virus Cyno 7 antiserum from a cynomolgus monkey 6 weeks after recovery from overt B virus infection; B virus Rh1 and B virus Rh3 antisera from the 2 rhesus monkeys 6 months after recovery from overt infection, and HSV T1 antiserum (from the author) which had the same neutralization titre as samples from the same source before the start of work on B virus. All antisera were inactivated at 56° for 30 min before use.

RESULTS

The supernatants from washings of oral swabs gave positive evidence of B virus in all 3 monkeys when titrated in hamsters, mice and VERO cell cultures. Rabbits given the same material subcutaneously showed typical skin lesions and paralysis. One monkey, Rh2, died soon after the oral lesions had healed but Rh1 and Rh3 recovered in a few days and showed no further symptoms during the following 6 months.

The ganglian explants produced cell outgrowths in a few days and some cells showed a cytopathic effect (CPE) within 6–7 days. A large number of fibroblast-like cells developed in these cultures and in later passages appeared to be typical fibroblasts. The CPE took the form of a few cytoplasmic masses containing several clusters of small nuclei and occasional giant cells. Samples of supernatants inoculated into VERO cell cultures induced the typical syncytial CPE of B virus as early as 22 h after addition. The same supernatants inoculated subcutaneously into young rabbits induced large skin lesions which appeared 4–5 days after inoculation, followed by leg paralysis after 6 days, and death. When the ganglion culture supernatants were assayed for infectivity in hamsters and mice the virus content per ml was higher (7·9 $\times$ 10^5 Hamster LD_{50}, 4 $\times$ 10^5 Mouse LD_{50}) than that recorded for the supernatants from oral swabs taken

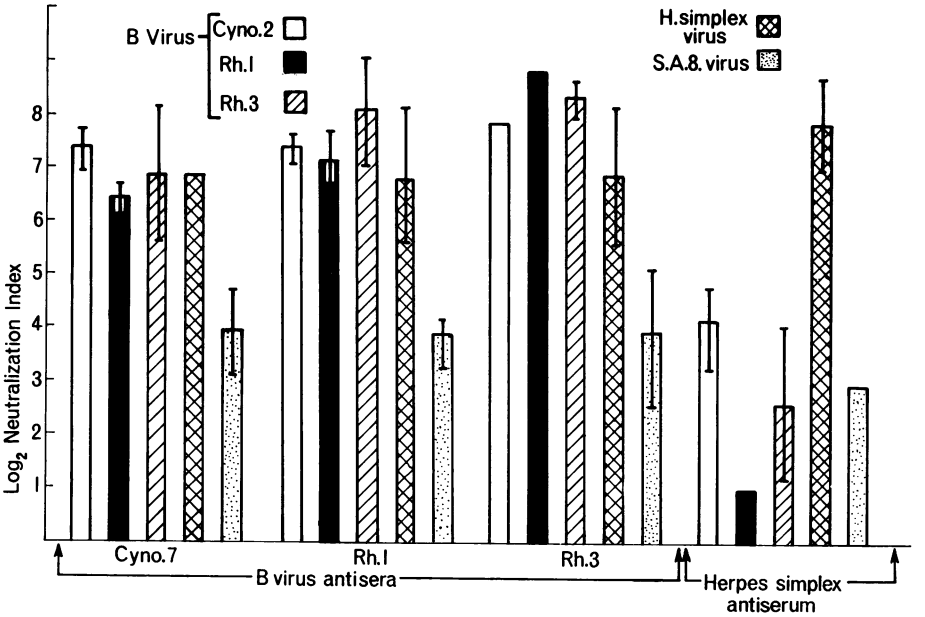

FIG.—Serological identification of B virus. Neutralization indices for 5 herpes viruses: Rh1 and Rh3 (derived from ganglia excised from rhesus monkeys 6 months after disappearance of overt symptoms) and 3 reference viruses. Tests made against B virus reference antiserum, herpes simplex reference antiserum and antisera from the 2 rhesus monkeys shortly before ganglion excision. 95% fiducial limits are shown where applicable.

at the time of overt infection ($3{\cdot}2 \times 10^4$ HLD_{50}, $7{\cdot}9 \times 10^2$ MLD_{50}), in spite of the few cells showing a CPE in the ganglian explant cultures.

The viruses obtained from the ganglion cultures Rh1 and Rh3, together with a Known B virus and a Known HS virus, were tested against the antisera specified above and the results are shown in the Figure. The ganglion culture viruses serologically resemble B virus Cyno 2 and the typical one-way antigenic relationship between HSV and B virus is shown by the failure of HS antiserum to neutralize any of the 3 B virus samples.

In addition, viruses isolated from oral swabs were similarly tested against the antisera; both were neutralized to the same extent as those isolated from the ganglion explants.

From their effect on tissue cultures and laboratory animals, as well as their serological typing, the two successive isolates from each monkey clearly represent the same virus.

The B virus from the original ganglion explant supernatant destroyed the cells from explant cultures at the 3rd passage. Up to the 8th passage ganglion cell cultures yielded no evidence of the presence of B virus. Both with and without a change of media, some cells showed spontaneous degeneration and regeneration, and in the former case supernatants were always tested for presence of B virus with negative results. After the 6th passage cells appeared to increase in growth rate as though a continuous cell line had been produced. At the 7th and 8th passage there was a predominance of small fibroblast cells, not unlike those in VERO cell cultures, and there were a few larger granulated cells with a stellate morphology.

DISCUSSION

The long suspected latency of B virus

in its natural host (the Old World monkey) has been confirmed, strengthening the possibility that activation of latent B virus infection, with shedding of live virus, occurs in simian hosts. Once again, the danger to humans handling monkeys which have recovered from a primary infection needs to be stressed. If the primary infection has passed unnoticed or has occurred before the animal enters quarantine a monkey will appear healthy and only become suspect if found to be seropositive to B virus, when a previous contact will be assumed. However, monkeys may recover from B virus infection without neutralizing antibodies being subsequently detectable (Vizoso, unpublished), a phenomenon which also occurs in rabbits (Vizoso, 1975*b*).

The use of live B virus to test for neutralizing antibody may itself involve a serious risk, as does the handling of infected monkeys. The antigenic relationship between B virus and HSV (see Figure) permits an alternative which, although less precise, is safe enough to be used in any laboratory. Positive reactions against HSV may lead to some mistaken inclusions among the seropositive group of monkeys identified for careful handling, but the likelihood of missing a potentially dangerous animal will be no greater than if B virus is used.

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