NOTES

A Virus Associated with Vulvitis and Balanitis in the Horse — A Preliminary Report

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A virus has been isolated from two outbreaks of a disease characterised by the development of lesions on the vulva of the mare and the penis of the stallion. The clinical findings appear to be identical to those described in the literature under such names as genital horse pox, coital exanthema and eruptive venereal disease (1, 2, 3). It is universally reported that this condition is due to a virus, however to our knowledge a virus has never previously been isolated.

In each case the infection involved both mares and stallions and appeared to be spread by coitus. Early lesions were vesicular or pustular in nature and occurred on the vulva and perineal region in the mares and on the penile mucosa of the stallions. Later the uncomplicated lesions appeared circular and pock-like, and as healing progressed affected areas became depigmented. The incubation period was 6 to 8 days and in the absence of secondary infection the lesions healed in 10 to 14 days. No effects on fertility were apparent but affected stallions were reluctant or refused to cover mares until lesions had healed.

The viral agent, which we prefer to leave unnamed until it can be classified was isolated by inoculation of horse kidney or testicle cell cultures with material taken by biopsy from lesions in infected mares. Within 24 hours of inoculation the cultures showed ballooning and rounding of cells and considerable cell death. Coverslip cultures stained by the May-Grunwald-Giemsa method showed multinucleated giant cells (polykaryocytes) containing up to 20 nucleii. Many of the nucleii in both giant cells and others showed intranuclear Type A inclusion bodies similar to those seen in herpesvirus infections. The virus was propagated in cultures of equine testicle, kidney, thyroid, adrenal and bone marrow cells but failed to multiply in primary bovine fetal kidney cells or in bovine kidney cell line cultures. In rabbit kidney cells only slight viral multiplication took place. In contrast the virus of equine rhinopneumonitis (EVR) will replicate well in all the above mentioned cells.

We have found that our virus like that of EVR is inactivated by ether and chloroform treatment and inhibited in the presence of 5-iodo-deoxyuridine, indicating it is a DNA virus. These features, along with the cultural characteristics of giant cell formation and development of intranuclear inclusion bodies suggest it may belong to the herpesvirus group.

Preliminary hemagglutination trials have shown EVR will agglutinate horse but not chicken erythrocytes, whilst our viral agent will agglutinate chicken but not horse erythrocytes.

We have conducted two transmission trials in horses. The first involved three mares, one of which was infected by rubbing a suspension of lesion material taken by biopsy from a natural case, into scarified vulvar epithelium. Lesions appeared in 8 days, and material taken from this animal was used to inoculate the other two mares in a similar way. One of these had previously experienced a natural infection of the disease and showed no evidence of reinfection. The other developed typical lesions.

In the second experiment three mares and a stallion were used. One of the mares received fifth-passage tissue culture-propagated virus. The second received a mixture of the virus and a hemolytic *Staphylococcus* isolated together with the virus from some of the field cases. The third received the *Staphylococcus* alone. The first two animals, which received virus, each

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showed typical lesions in 6 to 8 days but the third remained normal. The stallion was bred to the second mare during her period of active infection but failed to develop any visible lesions. He did, however, develop antibodies.

Serological studies carried out on sera from the second transmission trial demonstrated the development of low neutralizing antibody titres to the virus in the first two mares and in the stallion, the third mare remained negative. The three mares were negative for EVR neutralizing antibodies, both before and after infection. The stallion had a titre of 1:100 for EVR both before and after exposure to our virus.

Serological tests with rabbit sera prepared against our virus and EVR showed no evidence of cross neutralization. Several horse sera with neutralization titres up to 1:1600 against EVR had no effect on the growth of our virus in cell culture.

One of the studs from which virus was isolated suffered an outbreak of rhinopneumonitis abortion toward the end of gestation during the same breeding season. The virus of EVR was isolated from several of the aborted feti.

In conclusion, we believe that we have isolated in tissue culture on two occasions a virus not previously described which is a causative agent of so-called genital horse pox. Our investigations to date indicate that the virus is probably in the herpesvirus group, but differs immunologically and culturally from the virus of equine rhinopneumonitis. After consideration of the salient clinical features observed, equine venereal vulvitis or balanitis is suggested as the most suitable name for this disease.

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