lution. Apart from Aeppli's paper, polyploidy has not been reported previously in the family Dendrocoelidae. But in another group of fresh-water triclad, the family Planariidae, several examples of naturally occurring polyploidy are known. In some morphologically uniform species of this family a series of forms ('microspecies') exists with different chromosome numbers due to replication of the basic sets. This has occurred to such a degree that enneaploid and decaploid numbers have been recorded³.

Full details of the cytotaxonomical analyses reviewed above will be given elsewhere in the near future.

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VIROLOGY

Eclipse Phase of Vaccinia Virus

THE development of a plaque technique for titrating vaccinia virus on monolayers of chick embryo cells without an agar overlay¹ has furthered the investigation of cell-virus interaction in this system and has produced evidence of an 'eclipse phase' under certain experimental conditions. The essential prerequisite was that the inoculum, consisting of an elementary body suspension prepared from a rabbit skin-passaged strain of vaccinia by the method of Hoagland, Smadel and Rivers², was treated ultrasonically before use. Following the infection of monolayers by contact with this inoculum for 5 min. there was a rapid loss of infectivity of adsorbed virus, estimated to be probably about 30–40 per cent of the amount adsorbed, occurring within 10 min. from the first contact between virus and cells. Thereafter the rate of loss was slower, a further 70 per cent of the virus present at the end of the first 10 min. disappearing during 4–6 hr. The titre of virus in the culture began to rise at 7–8 hr. due to production of new virus.

At the period of lowest infectivity, after about 5 hr. of incubation, monolayers were dispersed by trypsin or ethylenediamine tetraacetic acid. The resulting cell suspensions were titrated as whole cells and after disintegration by ultrasonic treatment. Significantly higher titres were obtained with cells, the ratios being of the order of 15–30 : 1.

It was not possible to assess with confidence the quantitative difference in titre between whole and disintegrated cells since a number of possible fallacies inherent in the experimental methods have been demonstrated. Both trypsin and ethylenediamine tetraacetic acid caused the cells to shed about half their virus, and this was discarded when the cells were washed before titration. This virus would not have been removed by washing the monolayers or released spontaneously into the medium. There is no certainty that all the cells potentially capable of producing new virus would survive and do so when inoculated to another monolayer.

The low titre of disintegrated cells was not due to harming the virus by the ultrasonic treatment used to disintegrate them. Such treatment is an efficient method for releasing virus from cells but there is no

way of establishing that all the virus in cells is made detectable by this means. In titrating virus in disintegrated cells a dilution of at least 1 : 8–1 : 16 was required to obtain maximum plaque counts.

Taking these points into consideration, the results obtained appear nevertheless to indicate an eclipse phase as they demonstrate a marked fall in infectivity of a culture before the production of new infective virus and, at the stage of lowest infectivity, that cells which do not contain infective virus will ultimately produce it. The early and rapid loss of a considerable part of the infectivity of a culture, within a few minutes of first contact with virus, suggests that some virus is 'eclipsed' almost at once although the process continues for several hours. This probably indicates asynchrony in the cells of the monolayer. A similar time relationship for the escape of adsorbed virus from neutralization by antiserum has been shown and there is evidence that this escape indicates initiation of infection.

Similar evidence for an eclipse phase was not obtained when the inoculum was used without ultrasonic treatment. With this inoculum no significant loss of infectivity of adsorbed virus could be detected before appearance of new infective virus. A loss of about 70 per cent occurred in 8 hr. if the adsorbed virus was treated with antiserum before incubation and only the unneutralizable portion taken into account. Even so, attempts to demonstrate a difference in titre between whole and disintegrated cell suspensions from cultures of HeLa cells by pock counts at the phase of lowest infectivity were unsuccessful; both had about the same titre.

It appears, therefore, that ultrasonic treatment of an elementary body suspension affected the subsequent interaction between virus and cells in chick embryo cell monolayers. The treated virus was more uniformly capable of infecting cells and going into an eclipse phase. It caused a more rapid production of new virus and a greater yield. Why this should be so is not explained. The titre of an elementary body suspension was increased several times by ultrasonic treatment; and when monolayers that had adsorbed virus from an untreated suspension were disintegrated immediately afterwards, the amount of virus recovered by either plaque or pock titration was up to 10–15 times greater than the number of plaques that formed on similar monolayers incubated undisturbed with all their adsorbed virus. The simplest explanation would be that clumps of elementary bodies were dispersed by the treatment, but attempts to get microscopic evidence of it, by dark ground or electron microscopy, were unsuccessful.

The effect of ultrasonic treatment on virus may depend upon the nature of the virus preparation and its source. Preliminary observations suggest that the titre of a crude preparation of virus made by breaking up infected chorioallantoic membranes and removing the gross debris by centrifugation was not increased by the amount of ultrasonic treatment used for the elementary body suspensions.

A full account of this work will be published in due course.

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