A Simian Virus of New Morphology

(accepted 31 may 1967)

Cultures of monkey kidney tissue frequently exhibit spontaneous degeneration characterized by the formation of multinucleate giant cells which subsequently become vacuolated and assume a foamy appearance. The degeneration has been shown to be caused by a virus (1, 2). We describe the structure of a virus, strain MK 5, isolated from a batch of rhesus monkey kidney tissue culture which showed spontaneous foamy degeneration which was typical except that it progressed faster and became more widespread than is normally observed. On subculture in monkey kidney tissue culture free of foamy agent the usual type of foamy degeneration occurred. The virus was propagated once in rabbit kidney tissue culture and subsequently twelve times in HEp 2 cells where it formed syncytia similar to those formed by foamy agent in HeLa cells (1). The cytopathic effect of the virus in HEp 2 cells was best seen when cells were trypsinized several days after inoculation and subcultured into fresh tubes. The virus was ether sensitive, grew to low titre, i.e. 100 to 1000 TCD50 per ml., and even after incubation until the stage where all cells appeared to have degenerated the infectivity remained mainly cell-associated. Virus passed twelve times in HEp 2 cells on reinoculation into monkey kidney tissue culture caused the formation of typical foamy degeneration in 4 days. No haemadsorption was detected using guinea pig, human group O, monkey or chick erythrocytes. Unlike B virus (3), MK 5 was not pathogenic for rabbits by the intracerebral route. The virus when incubated at 42° showed a threefold increase in titre after 1 hr.; the infectivity subsequently decreased by 90% per 4 hr. period. The initial rise was probably due to partial dispersal of virus aggregates since preparations examined by electron microscopy indicated that the virus was usually found in large clumps. The source of virus for electron microscopy was either the original monkey kidney tissue showing spontaneous degeneration or infected HEp 2 cells. Cells + virus were added to 5 x 3/4 in. test tubes which were kept stationary for 18 hr and subsequently rolled at 37°. When most of the cells had degenerated the medium was replaced by 0.25 ml of water. The tubes were rolled for a further 30 to 60 min. and the supernatant fluids used directly to prepare grids for electron microscopy. Occasionally the cells were frozen and thawed once after rolling and the debris removed by centrifugation at 1000 rev./min. for 10 min. A drop of this preparation was placed on a waxed slide and a carbon-coated grid floated on the surface. The grid was removed after 5 to 10 min., blotted and allowed to dry. The preparation was stained with 4% phosphotungstic acid, pH 7.0. Alternatively the stain was mixed with an equal volume of the virus preparation and the mixture applied directly to a grid. All specimens were examined using an AEI electron microscope, type EM 6B. Several grids were prepared from each specimen since it was often necessary to examine more than one before a group of virus particles was seen. No virus other than the one described was detected in infected cells nor was any seen in the uninoculated HEp 2 cells. The morphology of the virus as seen in the original monkey kidney was unchanged after passage in HEp 2 cells.

When examined in preparations which were not dried before staining the virus
consisted of a roughly spherical body approximately 1050 Å in diameter (Pl. 1, fig. 1, 2). These bodies often appeared to become herniated when they dried in the presence of stain and this gave rise to shadowy structures which were associated with the original particle. The virus was covered by projections which often remained attached to the escaping contents (Pl. 1, fig. 1); they measured 150 × 50 Å and when seen end-on looked triangular.

When the virus was dried before staining it presented a quite different appearance. The outer envelope structure seen in undried preparations was usually penetrated by stain and an inner capsid was revealed (Pl. 1, fig. 3). These capsids measured 700 ± 50 Å (60 particles measured) and there was often more than one in a single envelope. Free capsids were often seen but their structure could not be established with certainty although the techniques used were adequate to resolve the substructure of a herpes virus capsid.

The relationship of MK 5 to foamy virus is problematical. Jordan, Plummer & Mayor described the structure of several foamy agents isolated from green-monkey kidney and rhesus-monkey testicular tissue (4). The morphology of these viruses indicated a probable relationship to the myxovirus group; they were quite different from the virus described here. Several strains of foamy virus were isolated by Johnston who divided them into two distinct serological types (5). More recently Stiles, Bittle & Cabasso added a third type on the basis of serological evidence (6). Clearly more than one virus type is capable of causing foamy degeneration of monkey kidney tissue cultures. The nature of the nucleic acid of MK 5 and its relationship to foamy viruses already described must await further studies, which would be much easier if more infectious virus could be produced than is possible at present.

REFERENCES

(Received 3 May 1967)

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EXPLANATION OF PLATE

Fig. 1 and 2. MK 5 virus negatively stained with potassium phosphotungstate. The virus was not dried before the addition of stain. Scale = 1000 Å.

Fig. 3. MK 5 virus dried before staining. Scale = 10000 Å.