We do not know the mechanism of this potentiating effect. It can be postulated that guanidine and 5-fluorouracil both interfere with the ribonucleic acid synthesis, or that 5-fluorouracil makes the cells less suitable for supporting the virus replication. However, Kovacs[4] has observed that HeLa cells treated with large doses of colchicine are able to support poliovirus replication, as do the untreated cells.

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## Intranuclear Inclusions produced by Bovine *para*-Influenza

Myxoviruses generally produce cytoplasmic but not nuclear inclusions in infected tissues. Reports on the cytopathology of human strains of the *para*-influenza viruses[1–6] in stained tissue culture preparations record only the finding of intracytoplasmic inclusions, although in *FL* cells specific intranuclear immunofluorescent staining has been demonstrated with *para*-influenza 3 (ref. 3). Reisinger *et al.*[7] reported both intranuclear and intracytoplasmic inclusions in calf kidney tissue culture infected with the *SF* 4 bovine strain of *para*-influenza 3.

We have passaged the *SF* 4 strain in primary monkey kidney tissue and *Hep* 2 cell cultures and have been able to demonstrate nuclear and cytoplasmic inclusions in both systems. Intranuclear inclusions have been most readily demonstrated in infected *Hep* 2 cell cultures. Cultures were grown on a medium consisting of Earle's BSS + 15 per cent horse serum + 0·1 per cent glucose + 1 per cent yeast extract + 0·2 per cent sodium bicarbonate with antibiotics. Before infection, cultures were changed on to a maintenance medium of the same composition as the growth medium, except that the horse-serum content was reduced to 1 per cent. Tube cultures inoculated with approximately 100 $TCID_{50}$ were fixed with Bouin's fluid at 2, 3, 4 and 6 days post-infection and were stained with Ehrlich's hæmatoxylin and eosin. Eosinophilic nuclear inclusions were first detected at 3 days, and at this stage were only in the nuclei, contained within syncytial areas. By four days the inclusions were demonstrable in all the nuclei within syncytial areas. Multinucleated cells containing nuclear inclusions always contained also cytoplasmic inclusions. One to nine inclusions were found scattered within the nucleus, and were sometimes of such a shape as to suggest that two or three had become fused together. Fig. 1 shows an infected *Hep* 2 cell sheet fixed and stained four days after infection. The clear halo surrounding the inclusions is probably a product of the method of fixation.

I have been unable to demonstrate similar nuclear inclusions in *Hep* 2 cells infected with a human strain of para-influenza 3 under the same conditions. These findings suggest a biological, as well as a serological[8–10], distinction between human and bovine strains, although further work is needed to demonstrate that nuclear inclusion formation is a constant distinguishing feature. Waterson[11,12] has suggested that the measles, distemper and rinderpest viruses be placed in the Newcastle disease virus group of myxoviruses. The production of nuclear inclusions by *SF* 4 virus, an accepted myxovirus, provides another link in this association. It also means that not

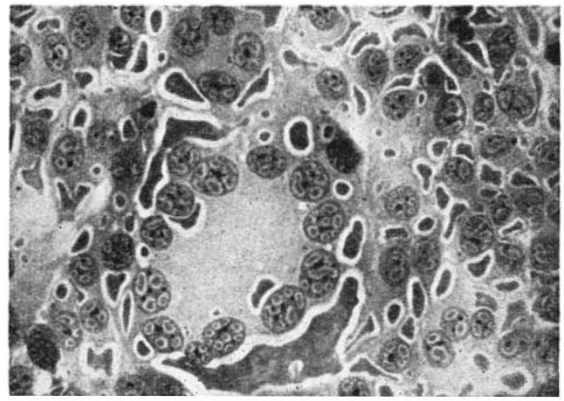

Fig. 1. *Hep* 2 cell monolayer 4 days after infection with *SF* 4 virus (stained with hæmatoxylin and eosin, × *c.* 233). Individual cells have lost their identity, becoming fused into a large syncytium. Nuclear and cytoplasmic inclusions are numerous and are surrounded in each case by a halo

every virus growing in monkey kidney and *Hep* 2 cells producing syncytia and nuclear inclusions is measles as is sometimes assumed. It might be easy to confuse the two without serological or hæmagglutination tests.

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## Demonstration of Leptospiral and Viral Antigens in Formalin-fixed Tissues

During the past few years the fluorescent antibody procedure has been applied with increasing success in the demonstration of microbial antigens. Naturally occurring cases of leptospirosis, venereal infections, influenza, rabies, and other diseases have been diagnosed[1] and further investigations will undoubtedly extend the application of this new diagnostic tool.

The application of the fluorescent antibody procedure is nevertheless limited by the lability of some of the antigens to be demonstrated. The quick freezing of tissue specimens, which is the best method for retaining the immunological activity of the antigens, is often not practicable under field conditions. Both fixation in 10 per cent formalin and storage at room temperature progressively decrease the property of the antigens contained in the tissues to bind with the fluorescent conjugate.

Application of the fluorescent antibody procedure to the detection of leptospiral[2] and viral[3] antigens in formalin-fixed tissues has been attempted with negative results. Its feasibility was considered worth investigating, and