several trials using identical assay hosts, CMV showed greater infection by the magnesium–phosphate complex than did TNV. Other preliminary evidence indicates that AMV in the presence of the complex is found to remain active longer than virus in phosphate. Therefore, magnesium may act by stabilizing the virus.

The purified preparation of TMV obtained from R. Reichle is gratefully acknowledged. This work was supported by grant *G*9820 from the U.S. National Science Foundation.

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Loss of the Hæmagglutinating Capacity of Fowl-plague Virus after Culture on HeLa Cells

Certain viruses undergo changes in their hæmagglutinating capacity, when propagated in certain cultures. Green *et al.*[1] and Mogabgab *et al.*[2] discovered, by examining strains of influenza virus, that the hæmagglutinating capacity is modified when passing from one cellular system to another.

Recently, Moscovici *et al.*[3,4] described the fact that some strains of ECHO virus lose their hæmagglutinating capacity when cultivated on HeLa cells, whereas they retain such capacity if cultivated on monkey kidney cells. Moreover, the virus which has lost its hæmagglutinating capacity does not regain it when cultivated again on cells of monkey kidney.

More recently, Johnson *et al.*[5] showed that loss of hæmagglutinating capacity occurs when Coxsackie *A*-21 virus is cultivated on cells of cancerous origin (HeLa and *KB* strains), whereas the capacity is maintained when cultivating the virus on primary human embryonic epithelial cells and semi-continuous diploid human embryonic fibroblastic cells.

It has been also demonstrated by Cassel *et al.*[6] that on serial passage in the Ehrlich ascites tumour loss of hæmagglutinating power occurs in three vaccinia virus strains. Tumorous ascitic fluid was removed from mice, and a cell-free tumour fluid with hæmagglutinin-inhibition power obtained.

We have observed the loss of the hæmagglutinating capacity of fowl-plague virus cultivated on HeLa cells, whereas it retains its capacity when cultivated on chick embryo cells.

The maintenance medium used is the same for both types of cells. It consists of: Hanks's balanced salt solution, 85 per cent; lactalbumin hydrolysate (5 per cent), 10 per cent; calf serum, 3 per cent; penicillin, 100 u/ml.; streptomycin, 100 mg/ml.; nystatin, 20 u/ml.; phenol red (0·2 per cent), 1 per cent; *p*H = 7·2. The cells are cultivated in hexagonal flasks of 'Pyrex' glass with a total capacity of 225 ml., containing 10 ml. of culture medium.

Starting from virus cultivated on chick embryo, 20 passages were performed with fowl-plague virus through chick embryo cells; the hæmagglutinating titre and egg lethal dose 50 per cent of each passage were determined.

It is possible to observe a rapid adaptation of the virus to the cellular culture, with stabilization of the evolution period of the infection, and appearance of cytopathic effects 24–48 h after inoculation of the virus.

The hæmagglutinating titre decreases from the first passage to the fourth, increasing afterwards up to a considerable value which is stable with small oscillations. The 50 per cent egg lethal dose undergoes a slight variation during the experiment, with an evolution apparently similar.

At the same time, 14 passages were made of fowl-plague virus on HeLa cells. For each one, samples were taken from the culture medium during the time of the passage; the hæmagglutinating titre and egg lethal dose 50 per cent were fixed. The adaptation of the virus is slower than in the preceding case; it becomes stable, but the evolution period of the infection is longer and the cytopathological effects appear later. The hæmagglutinating titre decreases rapidly, disappearing at about the third passage. However, the cytopathological effects continue to appear during all the following passages.

The existence of virus in the culture medium of infected HeLa cells was detected by electron microscopy; its morphological aspect is similar to that of the virus cultivated on chick embryo cells. Moreover, tests of complement fixation with antigen obtained from virus propagated on HeLa cells and with sera obtained from the same virus present a positive reaction reciprocally and in the presence of antigens obtained from virus propagated on chick embryo and chick embryo cells.

It has been shown that virus cultivated on chick embryo cells hæmagglutinates to a different extent from human type O, sheep and chicken erythrocytes, whereas virus cultivated on HeLa cells hæmagglutinates none of them.

Moscovici and La Placa[4] conclude that this phenomenon is due to an unknown virus-modifying factor, present in the human tumour cell and released at the time of infection, which causes a permanent modification of the surface of the virus. Johnson suggests the possibility that perhaps this is not an uncommon phenomenon among human enteroviruses, and that the loss of hæmagglutinating capacity is due to two types of infectious virus particles: positive and negative hæmagglutinating. When cultivated on malignant cells, they undergo a selection which favours the propagation of negative hæmagglutinating particles, whereas on non-malignant cells the selection is on the contrary.

Our experiments were performed in order to determine the possible existence of a hæmagglutination-inhibitory agent inside HeLa cells. Having dismissed the possibility of influence by the serum constituent of the culture medium (3 per cent calf serum)—since this is the same medium used for both types of HeLa and chick embryo cells—we now believe that we have discovered a new factor and are trying to determine its characteristics. This factor, obtained from free culture medium cell extracts, might possibly act inside the cells on the synthesis of virus hæmagglutinins, preventing their formation or otherwise neutralizing them afterwards.

This work was supported by research grant No. *FG–Sp*–122 from the U.S. Department of Agriculture.

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