washed free of medium, scraped off the glass and ultrasonically disrupted in a small volume of tissue culture medium. Cell debris was removed by centrifugation at 3,000*g* for 10 min and the virus then deposited at 40,000*g* for 30 min. The pellet containing the virus was dispersed by ultrasonic treatment in a small volume of distilled water and mixed with an equal volume of 2 per cent sodium phosphotungstate, *p*H 6·0. After standing at room temperature for 10 min, drops of the mixture were placed on carbon-coated 'Formvar' grids and examined in the Siemen's 'Elmiskop 1' electron microscope using double condenser illumination at 80 kV accelerating voltage and magnification, 80,000 times.

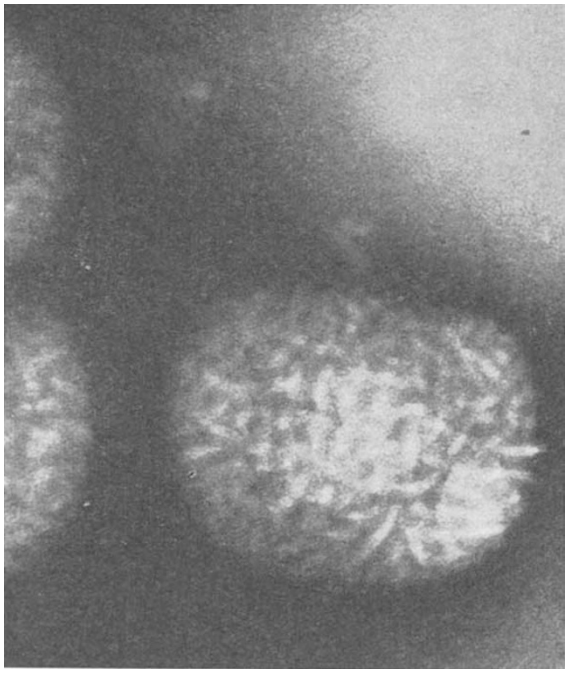

Fig. 3. Vaccinia; no PTA penetration (× 160,000)

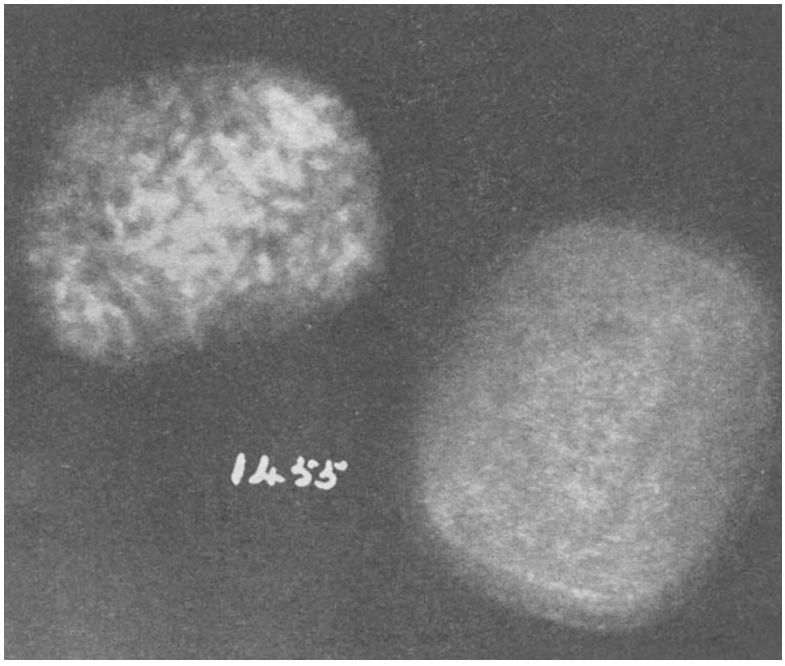

Fig. 4. Vaccinia; PTA has penetrated the particle on the right (× 160,000)

Figs. 1 and 2, showing the two types of particle observed, may be compared with Figs. 3 and 4, showing the corresponding morphological forms observed in similarly treated suspensions of vaccinia virus. Figs. 1 and 3 represent particles of virus into which the phosphotungstic acid (PTA) has failed to penetrate, the superficial beaded structure of the virus surface being clearly revealed. Enveloping membranes similar to the fragment seen in Fig. 1 are also frequently seen in vaccinia preparations. Figs. 2 and 4 show particles into which the stain has penetrated, revealing in each case a superficial 'capsule' of complex structure, surrounding a smooth inner body. Both types of particle have been reported in vaccinia virus preparations[4–6]. The details of their relationship and the significance of the fine structure observed in them will be the subject of a later communication.

In addition to the similarities in size, shape and fine structure revealed by negative staining, recent work (Fenner, F., personal communication) has demonstrated a fundamental serological relationship between the myxoma–fibroma group of viruses on one hand and the vaccinia–ectromelia group on the other. On the present evidence, therefore, the myxoma–fibroma viruses may be included with confidence as a sub-group of the pox viruses.

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Synthesis of Viral Ribonucleic Acid by a Chemically Inactivated Influenza Virus

It has been shown that the fowl plague virus, which belongs to the influenza *A* group, can be inactivated by an ethylene-iminoquinone (Bayer *A* 139, a gift of the Bayer-Werke, Leverkusen, Germany)[1] or by hydroxylamine[2,3] without losing its antigenic, neuraminidase, or hæmagglutinating activities. Bayer *A* 139 mainly reacts with the phosphate groups of nucleic acids, and in this way the sugar-phosphate 'backbone' is broken[4,5]. Hydroxylamine attacks the pyrimidines of the nucleic acids without breaking the RNA-strand[6]. Since during inactivation of the fowl plague virus with both compounds a phenomenon interpreted as multiplicity reactivation was observed[1,3], one should still expect viral RNA-synthesis in cells which were infected with partially or totally inactivated virus. Experiments designed to clarify this point are described here.

The methods applied for the determination of the biological activities are described in refs. 1 and 3. The amount of viral RNA mixed with normal newly synthesized cellular RNA was calculated from the shift of the oligonucleotide pattern of the RNA labelled with phosphorus-32 after RNase digestion of infected cells towards that of viral RNA[7,8]. The synthesis of normal cellular RNA is not inhibited by infection. Viral RNA-synthesis is an additional event[7,8].

Fig. 1 shows the inactivation kinetics of fowl plague virus with Bayer *A* 139 and

hydroxylamine, which is in accordance with former results[1,3]. If the partially or totally inactivated virus samples were added to chick fibroblast tissue cultures, the results presented in Fig. 2 were obtained. While the infectivity (plaque-forming units = PFU) and, to a lesser extent, the hæmagglutination titre (HA) drop quickly with increasing time of inactivation, the yield of viral RNA stays almost constant over a relatively long time. The oligonucleotide patterns of the 18-h points are identical with those of the controls, while the total radioactivity in the RNA (18 h) is, on the average, 10 per cent lower than in the controls. The complement fixation titre (CF), which measures the amount of the *s*-antigen, shows nearly the same dependence on the inactivation time as the viral RNA.

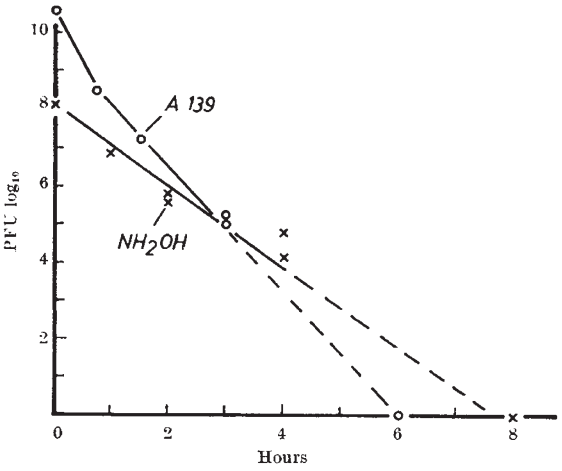

Fig. 1. Inactivation of fowl plague virus by Bayer *A* 139 and NH_2OH, respectively. Purified virus was inactivated by: (*a*) 1 per cent Bayer *A* 139 in 0·1 M *tris*-maleinate buffer *p*H 6·65; (*b*) 0·2 M NH_2OH in sodium chloride–phosphate buffer *p*H 7·0. Temperature, 21° C. For experimental details, see refs. 1 and 3. With higher inactivation it was not possible to determine the PFU exactly because of multiplicity reactivation (refs. 1 and 3). In those cases two points can be seen in the curves, which were calculated from two different dilutions

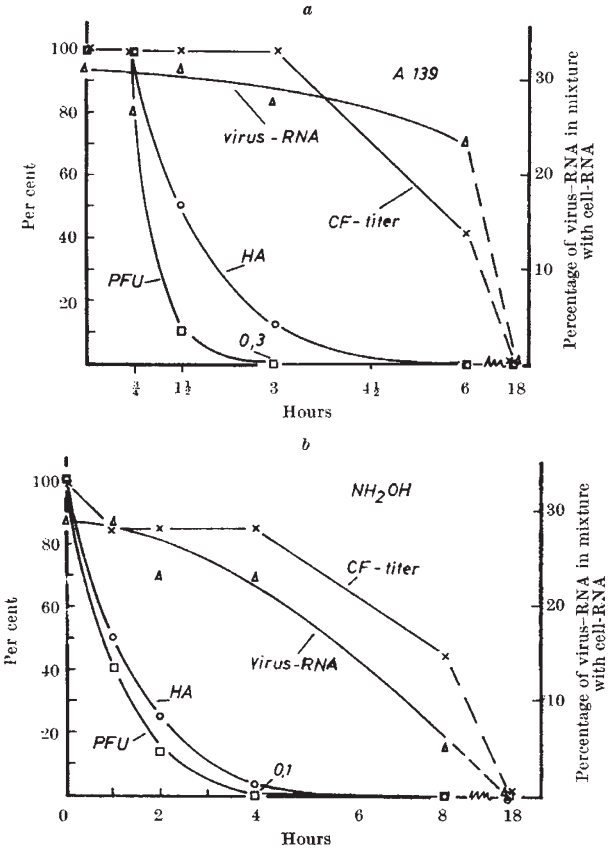

Fig. 2. Synthesis of virus-specific material in tissue cultures infected with partially or totally inactivated fowl plague virus. Chick fibroblasts were infected with virus samples inactivated by Bayer *A* 139 (*a*) or NH_2OH (*b*) for times as indicated at the abscissa of Fig. 1. On the left ordinate the percentage of these activities compared with controls infected with non-inactivated virus (= 100 per cent) is shown. The right ordinate represents the percentage of virus specific RNA in mixture with normal cellular RNA. For experimental details, see refs. 1, 3, 7 and 8

Although no material specific for this virus can be demonstrated in the infected tissue culture, if the virus is inactivated for a longer time (18 h or more), a high degree of interference has been found in these cells. Cells which were superinfected about 20 h after treatment with a virus preparation inactivated either with Bayer *A* 139 or hydroxylamine showed a virus yield 5–11 per cent of that of non-treated control cultures. This means that viral RNA-synthesis is not necessary for the interference phenomenon.

There is no significant difference between the activities of the virus samples treated for corresponding times with either Bayer *A* 139 or hydroxylamine. Therefore, it seems to make no difference whether the backbone of the nucleic acid is broken or only the pyrimidines are attacked. In both cases one has to assume that the viral RNA of the inactivated virus invades the cell and exerts there some activity even if the size of the RNA is lowered to some degree.

Prior to fowl plague viral RNA-synthesis the formation of 'early protein(s)' is necessary[8,9]. It may be that so long as that part of the virus RNA which codes for the 'early protein(s)' is not touched, the total amount of viral RNA is synthesized independently of some breaks or changes of the pyrimidines. If the total amount of the viral RNA is necessary for the formation of an infectious virus progeny, only a fraction of the virus RNA should code for the 'early protein(s)'. Because of the insensitiveness of the tests and some other uncertainties, like the relatively high multiplicity of virus particles per cell, even a rough estimate on the size of that fraction cannot be made. The hæmagglutinin has a rather complex structure of protein and carbohydrate. Therefore, it is conceivable that its formation is more sensitive to inactivation than the other activities (except PFU), which are assumed to be less complex.

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VETERINARY SCIENCE

Concentrating Ejaculated Sperm for Electron Microscopy

In previous work on the ultrastructure of bull sperm[1] semen samples cooled on ice during transportation to the laboratory were used. These samples were centrifuged immediately after arrival, but 24–48 h usually had elapsed from time of ejaculation to time of fixation of the specimens. Since various details of several of the membrane systems of the sperm were generally rather poorly pre-