were taken 3, 3½, 20 and 21 hr. after inoculation. The experiment was repeated several times. No local lesions appeared on the stripped area even 48 hr. after stripping, whereas about 150 local lesions appeared 23–26 hr. after inoculation on the rest of the leaf. On the other hand, bio-assays of areas devoid of epidermis revealed that virus was always present even after only 3 hr. In another experiment, portions of the epidermis of detached leaves were stripped off and 20 hr. later the stripped areas were then inoculated in the usual manner. No local lesions appeared on the stripped areas.

Stripping of the upper epidermis is very difficult, presumably because the epidermal layer is tightly connected to the underlying palisade cells. The strips were examined under the low magnification of a compound microscope and appeared to consist of the epidermal cells plus a thin portion of the palisade layer. The palisade layer was therefore rather heavily damaged in the stripping process.

It appears that in detached *N. glutinosa* leaves tobacco mosaic virus penetrates through epidermal strips into the palisade in less than 3 hr., but that for some reason under my conditions, the presence of epidermis is required for necrotic lesion formation.

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Utilization of Sheep Red Blood Cells in the Hæmadsorption Test for Myxoviruses

The hæmadsorption test[1,2] has been found to be a simple and useful means of detecting foci of infection by myxoviruses in tissue culture monolayers. The same response may be observed in cell cultures whether these viruses are non-cytopathic[3] or cytopathic. Briefly, the technique consists of decanting the growth fluid and covering the monolayer with a 0·4 per cent saline suspension of guinea pig or chicken red blood cells and allowing hæmadsorption to proceed for 10 min. at 4° C. After two saline washes, the areas of hæmadsorption are examined under low magnification.

Although divergent results have been reported on the ability of different myxoviruses to agglutinate sheep red blood cells[4–7] it was found in this laboratory that commercial sheep red blood cells (Brown Laboratory, P.O. Box 424, Topeka, Kansas) may be used effectively in the performance of the hæmadsorption test. Hæmadsorption was observed in a continuous cell line isolated from human kerato-acanthoma with the following egg-adapted strains of myxoviruses: influenza *A* (*PR*8, and *FM*1, *A*2) (Japan 305), *B* (Lee and *B*54), and mumps virus. Controls consisted of normal uninoculated cells and infected cells to which a 1 : 10 dilution of specific rabbit virus antiserum or normal rabbit serum was added 10 min. prior to the addition of sheep red blood cells. In all cases the controls showed no hæmadsorption. It was also possible to show hæmadsorption to sheep red blood cells with an influenza *C* strain in a continuous line of cells isolated from rabbit heart.

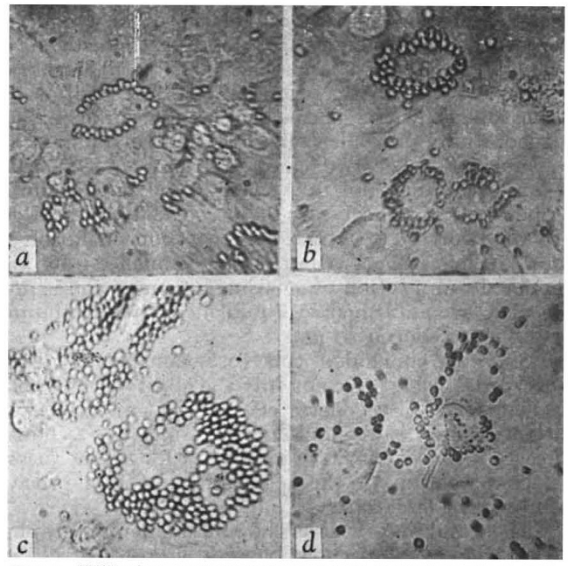

Fig. 1. Utilization of sheep red blood cells in the hæmadsorption test for myxoviruses

Fig. 1 shows hæmadsorption 'rosettes' seen with myxoviruses and the kerato-acanthoma cell line. Very little difference is seen between the response in the case of mumps virus and two of the influenza *A* strains (*PR*8, and *FM*1) tested (Figs. 1*a* and *b*). In the case of the *A*2 strain (Fig. 1*c*), however, the 'rosettes' often take on a much heavier appearance. The reason for this occurrence is not known, but it may be related to the amount of 'free' virus on the cell sheet. Distinct 'rosettes' are not seen as often with influenza *B* viruses (Lee, and *B*54) because of the toxicity and rapid destruction of the cells *per se* by these viruses (Fig. 1*d*).

The advantage of using sheep red blood cells for the hæmadsorption test is that they are readily accessible commercially and may be used for periods up to 3 weeks or longer when stored at 4° C.

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