

periods of activity. It further indicates that *Celerio lineata* is capable of distinguishing differences in thoracic temperature and of adjusting the heat output of the flight muscles during flight to compensate for changing external thermal stress.

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VIROLOGY

Mutual Precipitation of Two Viruses

SOME, but not all, pairs of proteins which are oppositely charged combine with and precipitate each other when mixed in salt-free solutions¹. The precipitates usually dissolve when salt is added, presumably because anions and cations of the salt substitute the negatively and the positively charged proteins, respectively, in the compound formed by electrostatic attraction.

Until recently it seemed improbable that pairs of viruses would precipitate each other by electrostatic attraction, for those examined mostly had iso-electric points near pH 4.5 with deviations from this value by less than two pH units. The possibility became evident with the knowledge that brome-grass mosaic virus (BMV) is iso-electric at about pH 8.0 (ref. 2) and satellite virus (SV) (ref. 3) at about pH 7.0 (Fig. 1). To investigate the possibility, tests were made to see whether these viruses would precipitate with tobacco mosaic virus (TMV), because there is a wide range of pH values at which they are positively charged and TMV is negatively charged (Fig. 1). At any pH value within this range BMV is considerably more charged than SV and thus appeared more likely to precipitate with TMV than SV.

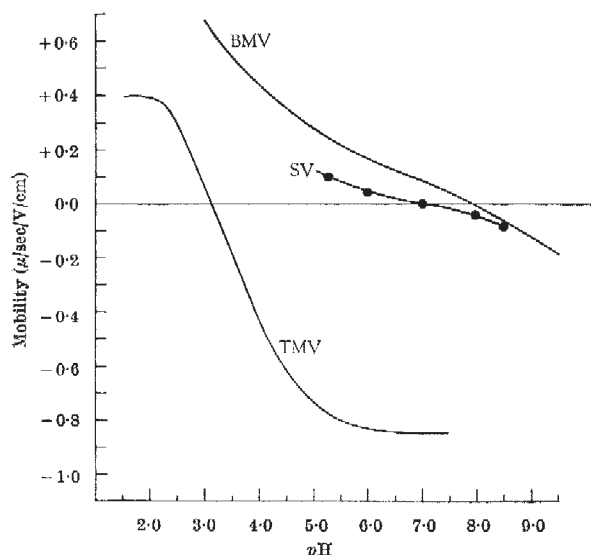


Fig. 1. Electrophoretic mobilities of TMV, BMV and SV at different pH values. The curve for TMV was taken from Kramer and Wittmann⁴ and that for BMV from Bockstahler and Kaesberg². They were obtained in buffers of ionic strength of 0.075 and 0.1, respectively. The curve for SV was obtained in the present work in M/15 phosphate buffer using a Perkin-Elmer electrophoresis apparatus. As isoelectric points and relative charge densities can depend on the presence of salt, any comparisons between TMV, BMV and SV, based on the curves shown above, apply only approximately to salt-free solutions.

When equal volumes of salt-free solutions of BMV and TMV at 1 g/l., adjusted to pH 5.0 with hydrochloric acid or sodium hydroxide, were mixed, a precipitate formed immediately, and when it had settled neither virus was detectable spectrophotometrically in the supernatant fluid. Precipitation occurred at all pH values between 4.0 and 6.5 and was most rapid at about pH 5.0. Adding sodium chloride to a concentration of 0.4 per cent or more immediately dissolved the precipitate.

By contrast, mixing salt-free solutions of SV and TMV in similar conditions did not produce a precipitate, and no interaction between the two viruses was detected when the mixture was examined in an analytical ultracentrifuge.

TMV and SV probably failed to interact because the charge density of SV was too small; at pH 5.3 (which is near optimal for precipitation between TMV and BMV) the electrophoretic mobility of SV (measured at 1 g/l. in M/15 phosphate buffer) is only about +0.1 μ/sec/V/cm, whereas that of BMV is about +0.3 μ/sec/V/cm. The mobility of BMV is slowed to about +0.1 μ/sec/V/cm when the pH is raised to just over 6.5, and at this pH BMV also fails to precipitate with TMV in spite of the fact that the raising of the pH slightly increases the negative charge density of TMV.

Salt-free solutions of SV and of yeast nucleic acid (1 g/l.) also failed to precipitate when mixed at pH 5.0, whereas solutions of BMV and of the nucleic acid immediately produced a precipitate that dissolved when sodium chloride was added to a concentration of 2 per cent. pH 6.0 was the upper limit at which a precipitate formed.

In salt solutions in which TMV and BMV do not precipitate one another they also fail to combine, for when a mixture of the two viruses in M/15 phosphate buffer at pH 5.3 was subjected to electrophoresis, the mobility of each virus and the size of the peak formed by it were the same as when it was used alone.

A suspension of the precipitate formed by mixing equal amounts of TMV and BMV caused numbers of local lesions on leaves of *Nicotiana glutinosa* similar to those caused by an equal amount of TMV inoculated alone.

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Inhibition of Virus Growth by a Toxic Factor from Asbestos Pad and Cellulose Acetate Membrane Filters

HOUSE¹ has shown recently that a factor which is toxic for baby hamster kidney cells (BHK 21, ref. 2) is eluted from asbestos pads by Eagle's tissue culture medium. This toxic factor inhibits to a marked degree the cloning efficiency of the clone U 13 which he used.

This observation by House appeared to be related to observations we had made previously during the preparation of a phosphate-low medium which we required for the growth of phosphorus-32-labelled foot-and-mouth disease virus³ in both pig kidney and baby hamster kidney cells. Using a formula based on Hanks's saline, but containing *tris* buffer instead of phosphate, we found in preliminary experiments that the yields of virus were the same as those in unmodified Hanks's saline. Unexpectedly, filtration of the phosphate-low medium through a Seitz *E.K.* asbestos pad resulted in a considerable reduction of virus yield. In view of House's observations we have now carried out further experiments to determine whether a toxic factor was responsible for the reduction in virus yields which we encountered in our preliminary experiments. Through Mr. House's co-operation we have been able to