virus purified in borate and phosphate against antiserum obtained by injection of rabbits with borate purified infectious virus. The borate preparations gave a strong precipitin reaction but the phosphate preparations gave little or no reaction.

As the phosphate method was known to be unsuitable for the preparation of the rod-shaped lettuce mosaic virus it was decided to compare the phosphate and borate methods for the purification of several viruses. Those chosen were the rod-shaped viruses causing lettuce mosaic, turnip mosaic, and potato mild mosaic (potato virus *X*), and the spherical viruses causing cucumber mosaic and arabis mosaic. The method was as follows. Two lots of tissue of similar infectivity were obtained by cutting infected leaves longitudinally. These were then homogenized (1·0 g of leaf per 1·5 ml. of buffer) in phosphate or borate buffer (0·5 M, *p*H 7·5) each containing 0·001 M sodium EDTA and 0·1 per cent thioglycollic acid. The subsequent purification procedure was the same as that described for lettuce mosaic virus except that the ultracentrifuge virus pellets were each resuspended in 0·5 ml. distilled water. The relative infectivity of the two types of final preparation at various dilutions was then determined by local lesion assay (or systemic infection in the case of arabis mosaic virus) after inoculation to *C. amaranticolor*. The results obtained with the five viruses are given in Table 1. All have been confirmed in several repeat experiments.

Table 1. Infectivity on *C. amaranticolor* of Five Viruses after Purification from Phosphate and Borate Homogenates

Virus and propagation host	Dilution of preparation	Mean No. of local lesions per half-leaf	
		Phosphate preparation	Borate preparation
Lettuce mosaic virus (lettuce)	undiluted	1	157
	1/10	0	57
	1/20	0	6
Turnip mosaic virus (turnip)	undiluted	2	286
	1/10	1	157
	1/20	0	24
Potato virus *X* (tobacco)	10^{-3}	6	48
	10^{-4}	3	10
	10^{-5}	0·1	7
Cucumber mosaic virus (tobacco)	1/10	250	142
	1/100	45	11
	1/500	42	1
Arabis mosaic virus (petunia)	10^{-4}	10/10*	10/10
	10^{-5}	2/10	10/10
	10^{-6}	0/10	1/10
	10^{-7}	0/10	0/10

* Denominator is the number of *C. amaranticolor* plants inoculated and the numerator is the number of plants afterwards with systemic symptoms.

As shown, the preparations of the rod-shaped viruses were of high infectivity when prepared in borate, but of very low infectivity when prepared in phosphate. By contrast, much smaller differences were obtained with the two spherical viruses, the infectivity of arabis mosaic virus being greater from the borate preparation and that of cucumber mosaic virus being greater from the phosphate preparation.

The foregoing infectivity tests were made only on the final preparations. To determine when, during the purification process in phosphate, the loss of infectivity of the preparations of the rod-shaped viruses occurred, samples were withdrawn at various stages and tested on the leaves of *C. amaranticolor*. The three viruses reacted similarly and it was found that although the original filtrates from the leaf homogenates in phosphate or borate buffer (containing sodium EDTA and thioglycollic acid) were equally infectious, those in phosphate had lost infectivity after clarification with butanol and centrifugation, whereas those in borate had not.

Possible reasons were considered for the loss of infectivity of the three rod-shaped viruses when butanol was added to the phosphate preparations. It was thought most likely that the virus particles had become aggregated and either were lost by removal in the early centrifugation or, if retained in the supernatant, were inactivated because they were aggregated. Alternatively, it was considered that they might have been disrupted by the butanol treatment. Electron microscope examination of the final preparations of the three viruses prepared both in phosphate and in borate, however, showed that neither explanation was correct. Both types of preparation contained equivalent numbers of largely unaggregated virus particles of normal size and shape. It thus appears that, following the phosphate clarification procedure, there was no change in the gross appearance of the rod-shaped virus particles although their infectivity was destroyed. Lettuce mosaic virus prepared in phosphate was also serologically inactive against antiserum prepared from infectious (borate-prepared) virus. The reasons for these effects are being further examined.

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J. A. Tomlinson

National Vegetable Research Station,
Wellesbourne, Warwick.

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MISCELLANEOUS

Microscopic Examination of Cell Monolayers grown in Plastic Bottles

Plastic culture bottles composed of a non-toxic polystyrene have been shown to be superior to polyvinyl or 'Pyrex' glass bottles for estimating the radiation sensitivity of cell monolayers cultured *in vitro*[1].

During the course of investigations involving the irradiation of monkey kidney cells grown on the surface of polystyrene bottles, it was observed that exposure of the bottles to toluol caused the dissolution of the plastic, leaving the intact monolayers floating free. This suggested a method for processing and examining the free monolayers.

The cell sheet adhering to the surface of the bottle is suitably fixed. The choice of fixative is important, as some (for example, Zenker's and Helly's) cause brittleness of the cell sheet with consequent fragmentation. Of the fixatives tried, the best results were obtained with Bouin's or Susa, although methanol or ethanol fixation for 24 h at 4° C appeared to be satisfactory. Alcohol-formalin may be used provided that prolonged washing is carried out prior to staining.

After fixation, a section of the plastic, the size of a microscope slide, is cut from the bottle by means of a hot knife and processed (washing, staining, dehydration, etc.) in ordinary staining dishes. The stained sections are immersed in toluol in individual coplin jars. Within a few minutes the plastic starts to soften, and with gentle shaking the monolayer is dislodged. As it floats free it may be teased on to a glass slide and a cover-slip applied for microscopic examination.

F. J. Stanfield
M. E. Lyman

Defence Research Kingston Laboratory,
Kingston, Ontario.

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