Chromatography of Tobacco Mosaic Virus (TMV) on Chitin Columns

While performing experiments on infected tobacco plant fibre to determine the virus-releasing enzymic activity of crude mushroom extracts (Townsley, P. M., unpublished results), it was noted that added chitin strongly adsorbed tobacco mosaic virus. At least a portion of the adsorbed virus could be released with concentrated phosphate buffers. This investigation describes the chromatographic properties of chitin for TMV.

90 gm. of chitin (Eastman Organic Chemicals) was reduced to a fine powder in a porcelain–'Carborundum' ball mill, in about 18 hr. The powder was suspended in 1 litre of *N* sodium hydroxide, stirred for about 0·5 hr. and collected in a Buchner funnel. The chitin pad was washed thoroughly with distilled water and then resuspended in 1 litre of 2 *N* hydrochloric acid. After settling for at least 0·5 hr. the top milky layer was decanted and discarded. The sediment was collected on a Buchner funnel, washed first with water, then with 95 per cent alcohol, and finally with ether.

10 gm. of prepared chitin was suspended in 250 ml. of water, and allowed to settle for 20 min. The top layer was discarded. The sediment slurry, now containing approximately 5 gm. of air-dried chitin, was poured into a chromatographic column, to form a column 17 × 1·3 cm. The column was washed with 25 ml. of 1 *N* hydrochloric acid, followed by 50 ml. of water, then 150 ml. of 0·1 *M tris*–hydrochloric acid, *p*H 6·8, and finally equilibrated with approximately 75 ml. of 0·01 *M tris*–hydrochloric acid, *p*H 6·8 (similar equilibration of the column with phosphate buffer, *p*H 6·8, prevented the column from adsorbing all the added TMV). The eluate from the column was approximately *p*H 6·8.

0·5 ml. of tobacco mosaic virus solution containing 0·3–2 mgm. of virus in 0·01 *M* sodium phosphate buffer, *p*H 6·8, prepared by two alternate cycles at 6,000*g* and 78,000*g* (ref. 1), was added to the column and the virus washed into the column with three successive 1-ml. aliquots of 0·01 *M tris*–hydrochloric acid, *p*H 6·8. 3 ml. of the latter buffer was then placed on the column and the column connected for linear gradient elution. The linear gradient progressed from water to 0·5 *M* K_2HPO_4. The eluate was collected in 4·3-ml. aliquots at 10-min. intervals in a fraction collector equipped for continuous optical density recording at 254 mμ.

As measured by optical density the virus was almost wholly accounted for in the two eluted peaks (Fig. 1). The results were reproducible, but the amount of virus in each peak varied. No explanation is offered for the appearance of two major peaks rather than one as might have been expected from the virus purified by ultra-centrifugation.

Material from both the peaks was birefringent and infective. Preliminary, comparative infectivity tests directly on elution, using six half-leaves of the local lesion host, *Nicotiana glutinosa* L., for each of the peaks, indicated that the material eluted at *p*H 7·2 was from four to ten times as infective as that eluted at *p*H 7·7. Spectrophotometric measurements gave a 260/280 mμ ratio of 1·23 and 1·29 respectively, for the peaks in the eluting solvent.

As a chromatographic agent for TMV chitin would appear to be very useful in view of the mild treatment required for adsorption and elution of the virus.

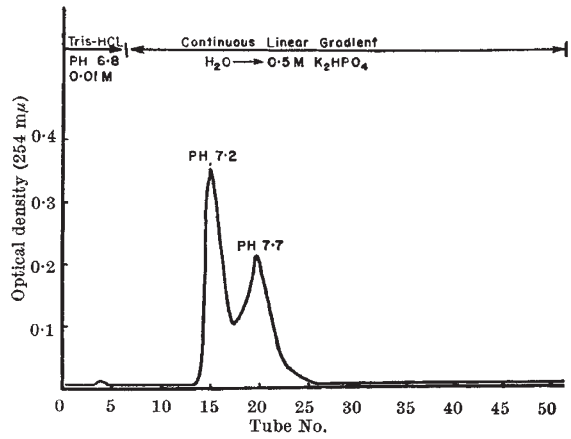

Fig. 1. An elution curve of tobacco mosaic virus from a chitin chromatographic column

Preliminary experiments have also shown that chitin may be used with similar ease for nucleic acid chromatography.

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[1] Townsley, P. M., *Canad. J. Biochem. Physiol.*, **37**, 1025 (1959).

CYTOLOGY

Peripheral Position of Sex Chromatin

In examining buccal smears for sex chromatin, one finds a surprisingly high incidence of sex chromatin bodies not merely at the nuclear membrane but also more precisely at the periphery of what appears to be a disk-shaped nucleus. The following discussion will provide evidence that, as in tissue cultures[1], the sex chromatin is characteristically at the edge of the nuclear disk in squamous cells *in vivo*. In establishing this let us first consider that squamous nuclei *in vivo* are flattened. This may be seen in mucosal smears when a mucosal fragment is folded over (Fig. 1). It is evident, too, that the cells in fragments in the smear maintain the same relative position to one another that they had *in vivo*, and that one is seeing the cells as one would have seen them in looking directly down on to the mucosal surface. Further, in most nuclei which can be reliably scored,

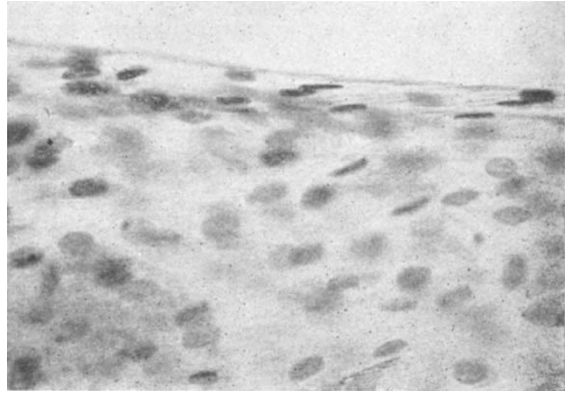

Fig. 1