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Separation of Poliovirus and Poliovirus RNA on Sephadex G 200*[1]

(Brief Report)

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With 1 Figure

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Poliovirus RNA has been chromatographed previously. *Kubinsky* and *Koch* (1) using methylated serum albumin on celite as column packing separated polio RNA from cellular RNA. *Lamb* and *Dubes* (2) using a column of hydromagnesite separated poliovirus RNA from intact virions, cellular RNA, DNA, and glycogen. Both methods are based on ion exchange; a gradient with increasing ionic strength is used to elute the substances adsorbed to the column. Gel filtration does not require an eluent gradient and the eluent can be chosen according to the necessities dictated by the further use of the fractions. In the case of virus and RNA fractionation phosphate buffered saline (PBS) was used as eluent because the infectivity residing in the various fractions could be determined on monkey kidney tissue cultures (MKTC's) without further adjusting the fractions to physiological conditions. The Sephadex gels separate substances primarily on the basis of differences of their molecular weight (MW) but also on differences in their structual configuration or chemical compo-

* Sephadex: Registered trade name, Pharmacia Uppsala, Sweden.

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sition. Substances with a MW greater than 200,000 are supposed to be excluded from the mycell structure of Sephadex G200 and consequently should flow freely, without separation through the gel. Hence it was not necessarily expected that a separation of poliovirus RNA (MW $1.7 \cdot 10^6$) and the virion (MW $4.6 \cdot 10^6$) would be possible (3).

Poliovirus antigenic type I (Brunhilde strain) was propagated in secondary MKTC's grown in 50 mm. petri dishes. After infection had destroyed most of the cells (cytopathic effect 4^+) the culture fluid was harvested and freed from cell debris by low speed centrifugation. The virus suspension was freed from soluble proteins by collecting the virus in a pellet (Spinco Model L Centrifuge) and resuspending the pellet in PBS.

Infectious RNA of poliovirus was prepared using essentially the method described by *Gierer* and *Schramm* (4). The purified virus suspension was shaken for 3 minutes with water saturated phenol. The phenol and water phases were separated; the phenol remaining in the water phase was extracted with ether. The column, which had an inner diameter of 1×110 cm. (final column bed 1×105 cm.) was prepared with Sephadex G200 which was previously swelled in PBS containing 2% phenol. Phenol was added to keep the column packing sterile. The column was then washed with sterile PBS lacking magnesium (PBS-A) in order to remove the phenol solution. The samples (2—3 ml.) were then applied to the column and eluted with PBS-A at room temperature. No pressure was applied to the column and the flow rates varied between 8 and 24 ml. per hour but no influence on the elution patterns resulting from changes in flow rates were observed. Fractions of 4 ml. were collected (G. M. E. Fraction Collector Model V15, Middleton, Wisconsin). The void volume (V_0) of the columns was determined with india ink; the carbon particles of the ink are too large to penetrate the Sephadex gel and therefore are eluted with a K_d value of 0. The inner volume (V_i) of the columns was determined with phenolsulfonphtaleine (phenol red, MW 354.37) as indicator. A substance of this MW penetrates the gel structure entirely and has a K_d value of 1.0. The concentration of virus or virus RNA respectively, accumulated in the various fractions was determined using the plaque technique on MKTC monolayers (5). To determine the amount of infectious RNA the facilitation procedure described by *Dubes* (6) was used. In experiments where mixtures of virions and RNA were chromatographed the infectious particles in each fraction were also tested for their RNase lability to determine whether they consisted of virions or viral RNA. Typical elution patterns of polio virions, virus RNA and a mixture of the two are shown in Fig. 1. The virions are eluted immediately after the void volume of the column which was determined with india ink to be 24 ml. Their K_d value consequently is 0.00. The peak of eluted virus-

RNA appeared in fraction 13, e. g. after 52 ml. of total elution volume (V_x). The total inner volume (V_i) of the column was 64 ml., as determined by the volume, after which phenol red was eluted (88 ml.) minus the void volume of the column. The K_d value for the polio RNA can be calculated after the formula $K_d = \frac{V_x - V_0}{V_i}$ which gives a value of 0.436.

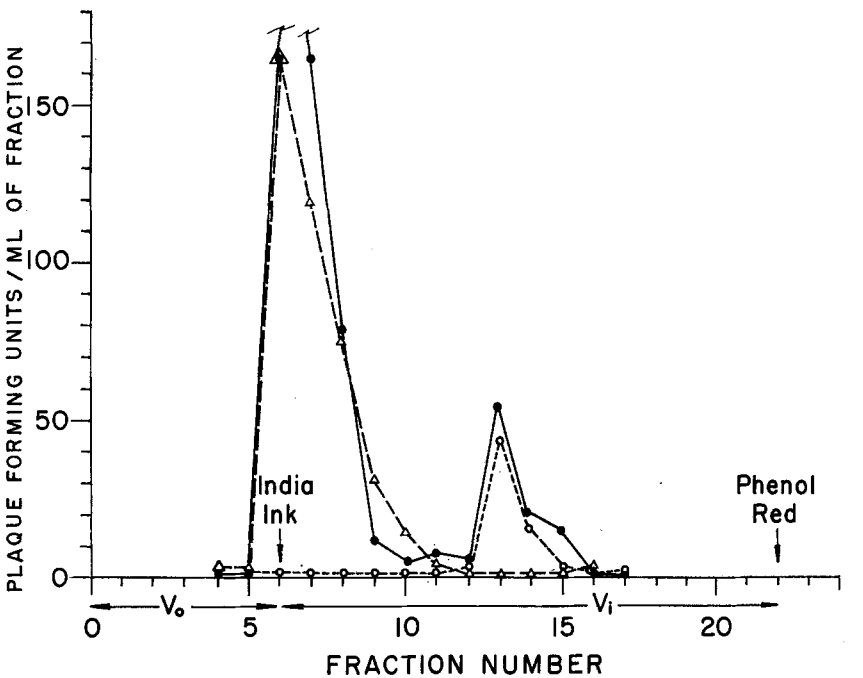

Fig. 1. Elution diagram of poliovirus and poliovirus RNA on Sephadex G 200. PBS-A was used as eluent.

△ – – – △ elution pattern of purified virus.
○ - - - - - ○ elution pattern of virus RNA.
●———● elution pattern of a mixture of virus and RNA.

The arrows point out the fractions where india ink and phenol red were eluted from the column respectively; these points determine the void volume (V_0) and inner volume (V_i) of the column. Each fraction comprised 4 ml. eluent.

Fig. 1 shows that a satisfactory separation of virions and viral RNA can be achieved with Sephadex G200. On Sephadex columns of smaller mycell structure (G100, G75) no such separation was possible. The RNA as well as the virions eluted with a K_d value of 0.00, a value reported for other high molecular RNA's (7). Phenol extracts prepared identically to infectious RNA but from uninfected cells were also chromatographed on Sephadex G200 gels and the elution pattern of material adsorbing UV-light at 260 mμ was studied. For reasons of clarity the elution pattern of UV adsorbing material is not shown in Fig. 1. Most of the phenol extrac-

table cell materials had a K_d value of 0.00 and no peak of UV adsorbing material was eluted at the position where viral RNA usually elutes from the column. The sensitivities of the spectrophotometrical assay for cellular RNA and the very sensitive plaque assay for infectious virus RNA are of different magnitudes; therefore, no quantitative comparison can be made, however, the results still suggest a separation of viral RNA from at least the bulk of cellular RNA on Sephadex G200.

As mentioned above, particles with a molecular weight above 200,000 are excluded from Sephadex G200 gel. Therefore in the case of poliovirus and virus RNA the separation which occurred cannot be attributed only to differences of the molecular weights of the virion and the RNA because both are above the exclusion limit of the gel. The results suggest the alternative that the chemical or structural configurations of the RNA are responsible for its resolution from the virion on the gel. If the resolution should be based primarily on structural differences chromatography on Sephadex G200 might separate single and double stranded RNA's.

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