CHROMATOGRAPHY OF INFECTIOUS RIBONUCLEIC ACID ON CALCIUM PHOSPHATE COLUMNS

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Infectious ribonucleic acid (RNA) extracted from Krebs 2 ascites tumour cells infected in vitro with encephalomyocarditis virus (Huppert & Sanders, 1958) is diluted considerably with cellular RNA. An attempt has been made to separate the infectious species from the bulk of the cellular RNA by adsorption chromatography on calcium phosphate columns. The results so far obtained suggest that the unit of RNA responsible for infection may be aggregated in solution. Continuously agitated cell suspensions were infected in vitro at a multiplicity of 3 (Sanders, 1957), controls receiving phosphate-buffered saline (PBS) instead of virus. Infected and control cultures were harvested at 6 hr. when the first new extracellular virus is found, at 18 hr. when virus release is already complete, and at 24 hr. 6 hr. cells were washed with PBS and resuspended in 0·02 M phosphate buffer, pH 7·2 containing nickel ions. The optimum nickel concentration needed to stabilize RNA infectivity is 1 Ni^{++} ion per nucleotide, a value already demonstrated for TMV RNA. (Cheo, Friesen and Sinsheimer, 1959). At the two later stages RNA was extracted from the whole culture to which nickel was added to the optimal concentration.

All samples were stored at -20° until extracted at 4° with phenol saturated with water containing nickel. Phenol was removed from the aqueous RNA extract with ether and the RNA ethanol-precipitated, washed 3 times with 67% ethanol -33% 0·14M sodium chloride, and redissolved in 0·14 M sodium chloride containing nickel. The resulting solution was

cloudy with nickel phosphate but was used directly. Such RNA preparations retained their original infectivity titre for at least 14 days at 4°, and for at least 24 hr. at room temperature.

Calcium phosphate columns were prepared according to the procedure described by Main & Cole, (1957). RNA was eluted at room temperature by a linear gradient from 0·005 M to 0·2 M phosphate buffer. A typical run lasted about 18 hr. Over 95% recovery was achieved and about 3 mg. RNA per ml. of packed column could be accommodated.

The concentration of RNA in the eluate was determined by optical density measurements at 260 mμ, and the phosphate molarity of the samples by the method of Allen (1940).

Infectivity tests of eluates were carried out in mice as described by Huppert and Sanders (1958).

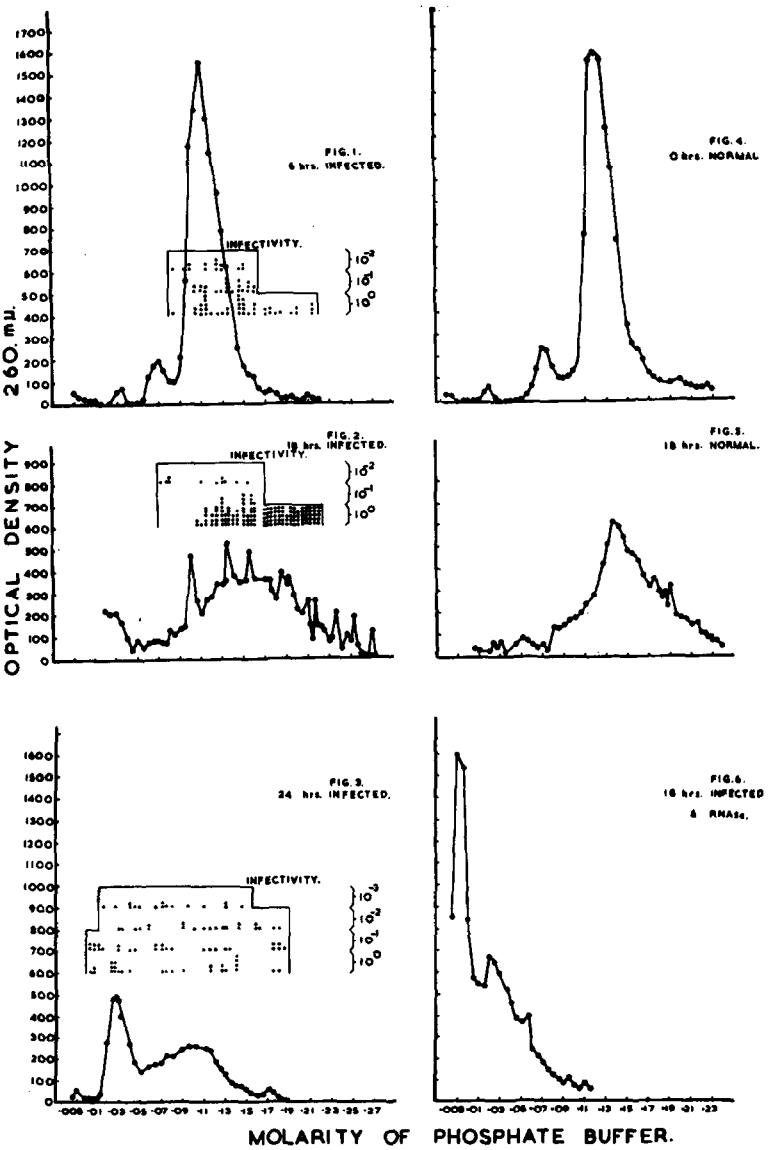
RESULTS

Figs. 1 and 4 illustrate the elution diagrams of RNA from 6 hr. infected and 0 hr. non-infected cells, respectively. No differences could be detected between the chromatographic profiles of such RNA samples in the early stages of infection indicating that the RNA component responsible for infectivity must be present in relatively low concentration. The difference between the profiles of RNA from 6 hr. and 18 hr. uninfected cultures, shown in Figs. 4 and 5 is probably due to deterioration of the cells.

In the presence of virus the profile of RNA at 18 hr. was further altered probably as a result of cell death (25% dead cells)(Fig. 2). These changes were still more evident in RNA from 24 hr. infected cells (50% dead cells)(Fig. 3).

Infectivity was present in samples eluted over a wide range of molarity (Figs. 1, 2, and 3). However, those eluted between ·15 and ·17 M phosphate had the most rapid lethal action. Infectivity could not be correlated with RNA concentration.

RNAse (1 μg per ml for 30 minutes at 37°) acting upon an 18 hr. infected sample (Fig. 2) completely destroyed the infectivity and greatly



altered its chromatographic profile (Fig. 6).

A curious feature of the work was that mice receiving diluted samples eluted in the region of 0·1 to 0·12 M phosphate died at least 24 hours earlier than those receiving the undiluted RNA suggesting some form of inhibitor is eluted in this region and that it can be diluted out.

DISCUSSION

It is a reasonable assumption that a complete molecule of EMC RNA is required before infectivity is apparent; however, it is not yet

established whether individual virus particles contain one or more such molecules. Since chromatography separates different molecular species, and since infective units are eluted over practically the whole chromatographic profile, the infective unit must be associated with more than one kind of molecular species. It may be suggested these different molecular species arise due to either (1) Aggregation of RNA molecules (Fraenkel-Conrat, 1957) which therefore are eluted at a phosphate buffer molarity corresponding to the sum of the molecular weights of the aggregated individual units; or, (2) phenol deproteinization could be incomplete and RNA may have small peptides attached (Takahashi, Karler & Knight, 1958; Habermann, 1959), which do not affect the molecular weight of RNA to any great extent but which could affect the adsorption of RNA by calcium phosphate. If similar sized infectious units have different sized peptides attached they may be eluted by different phosphate molarities.

The following evidence suggests chromatography on calcium phosphate separates RNA on a molecular weight basis and hence adds support to the aggregation hypothesis.

The dependence of molecular weight on molarity of eluant is shown by premature elution of low molecular weight protein (Tiselius, Hjerten & Levin, 1956), degraded DNA (Main & Cole, 1957) and degraded RNA (See Fig. 6). Since the infectious unit elutes over a wide range of molarity, it is suggested this unit is associated with different molecular weight components. Further, preliminary work with the Ultracentrifuge suggests that at least two components of different molecular weights exist in RNA of Krebs ascites tumour cells prepared by the phenol method. These appear to correspond to the two main peaks in the chromatographic profile of Fig. 1 and have infective properties (to be published).

Work is now in progress attempting further correlation of the schlieren patterns with chromatographic profiles, to break down the aggregates, and to elute the infectious unit over a narrow range of molarities.

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