GEL FILTRATION OF NUCLEIC ACIDS ON PEARL-CONDENSED AGAROSE [1]

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The use of gel filtration to separate molecules with molecular weights above $2 \cdot 10^5$ was made possible by the introduction of granulated agar gels by Polson (1961). These gels might allow separation of high molecular nucleic acids according to size. An artificial mixture of T2 DNA and E. coli RNA has been separated on granulated 1,5% agarose gel (Boman and Hjertén, 1962). The low flow rates in gels with agar concentrations less than 3% made them difficult to use but this was overcome by the introduction of pearl-condensed agar or agarose (Bengtsson and Philipson, 1964, Hjertén, 1964).

The present study describes gel filtration of nucleic acids from KB cells and poliovirus RNA on pearl-condensed 2% agarose. It is also shown that the composition of the buffer influences the elution pattern of poliovirus RNA.

Agarose preparation. Agarose was prepared from Special agar Noble (Difco) by the method of Russel et al. (1964).

Pearl-condensing. A hot solution of 2% agarose in water was pearl-condensed according to Bengtsson and Philipson (1964). The pearl-condensed agarose was sieved and the fraction 60 - 100 mesh (U.S.sieve series) was collected.

Poliovirus RNA. Poliovirus type 1 (strain E206) was grown, purified and extracted with phenol as described previously (Öberg et al., 1965). The virus was labelled with ^{32}P, since the amount of poliovirus RNA was too small to be mea-

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sured by UV-absorbancy.

KB-nucleic acids. KB cells were grown in spinner cultures in Eagle's MEM spinner medium (Eagle, 1959) with double strength of amino acids and harvested by centrifugation. The nucleic acids were extracted from the cell pellet by the phenol-duponol method (Philipson, 1961).

Gel filtration. A glass-column, 1.8 x 45 cm, with a sintered glass disc was packed by allowing a suspension of pearl-condensed 2% agarose to sediment in the column. The column was then washed with 500 ml of buffer before use. All buffers contained 0,5% butanol to prevent bacterial contamination. The experiments were performed at room temperature with a flow rate of 2 ml/cm^2/hour and fractions of 2 ml were collected. About 1 ml of the nucleic acid preparation was applied in each experiment.

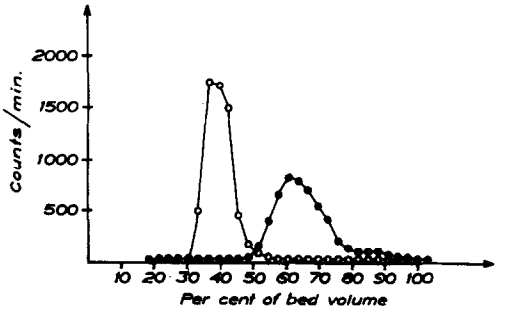

Fig. 1. Gel filtration of poliovirus RNA labelled with ^{32}P on a 2% agarose column, 1.8 x 26 cm, at room temperature in different buffers. The flow rate was 2 ml/cm^2/hour and 2 ml fractions were collected. The buffers were 10^{-3}M lithium phosphate, pH 6.0 (open circles) and $2 \cdot 10^{-2}$M lithium phosphate pH 6.0 with $2 \cdot 10^{-3}$M $MgSO_4$ (filled circles)

Results and discussion. Fig. 1 shows two experiments performed on the same 2% agarose gel column with the same poliovirus RNA preparation, but with two different eluting buffers. In 10^{-3} M lithium phosphate buffer, pH 6.0 poliovirus RNA, which has a molecular weight of about 2×10^6, elutes with the void volume and is thus not retarded. In 10^{-2}M lithium phosphate buffer pH 6.0 with 10^{-3}M $MgSO_4$ the RNA eluted at 60% of the bed volume. The reason for this is probably the tendency of RNA to assume different conformation in the two buf-

fers. According to Spirin (1964) high molecular weight RNA forms rods in solutions of relatively low ionic strength at room temperature. These rods are transformed to more compact coils in higher ionic strength and also in the presence of divalent metal ions as magnesium. Since gel filtration separates molecules of different size, but not necessarily of different molecular weight, variation in ionic strength and composition of the buffer gives a possibility to separate nucleic acids of the same size, but with different tendency to form coils or rods. It should therefore be possible to separate a double stranded helical form of DNA or RNA from a single stranded form with the same molecular weight, the single strand beeing more easily coiled in a compact form, when raising the ionic strength or adding magnesium ions. Preliminary results with double stranded poliovirus RNA support this hypothesis.

The recovery in experiments with poliovirus RNA was usually around 100%, measured as radioactivity, but in some experiments all RNA was adsorbed to the column. The reason for this is not clear, but since the amount of poliovirus RNA applied to the column was in the order of 0.05 µg, some charged groups in the agarose could be responsible for the adsorption. When poliovirus RNA mixed with KB-nucleic acids was chromatographed a quantitative recovery was always obtained.

A mixture of KB-nucleic acids and poliovirus RNA was filtered through a column of 2% agarose using $2 \cdot 10^{-3}$M sodium phosphate buffer pH 6.0 with 10^{-3}M $MgCl_2$ as eluant. As shown in Fig. 2 the KB-nucleic acids were separated into three classes. The first UV peak is DNA, the second ribosomal RNA's and the third transfer RNA's as determined by the diphenylamine reaction for DNA (Burton,1956), the orcinol reaction for RNA (Kerr and Seraidarian, 1945) and analytical ultracentrifugation. The ribosomal RNA's are thus not separated in two classes as might be expected. The reason for this is unclear, but the degree of coiling might be different in the two classes. Poliovirus RNA elutes between DNA and the ribosomal RNA's. The recovery is 80 - 100%, counted as radioactivity and UV absorption. The position of poliovirus RNA indicates that high molecular RNA's

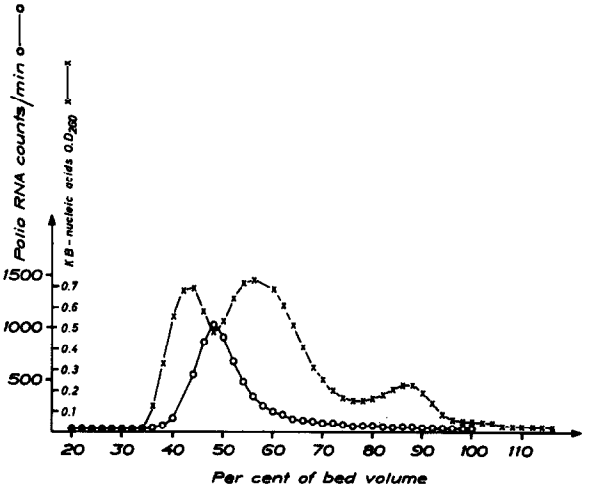

Fig. 2. Gel filtration of a mixture of poliovirus RNA labelled with ^{32}P and KB-nucleic acids, on a 2% agarose column, 1.8 x 40 cm, at room temperature. The flow rate was 2 ml/cm^2/hour and 2 ml fractions were collected. The buffer was $2 \cdot 10^{-3}$ M sodium phosphate pH 6.0 with 10^{-3} M $MgCl_2$.

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could be separated from each other. To achieve a complete separation a longer column would be necessary. Experiments on pearl-condensed gels with varying agarose concentrations have shown the separation effect of 1% agarose gels to be superior to that of 2% gels for nucleic acids with molecular weights above $5 \cdot 10^5$. On 4% gels DNA and ribosomal RNA's elute together in the void volume, but the transfer RNA's are well separated from the other nucleic acids. As pointed out above the separation is highly dependent on the buffer composition. To conclude it has been shown that gel filtration on pearl-condensed agarose gels provides an additional tool for separation of different classes of nucleic acids.

REFERENCES

Bengtsson, S. and Philipson, L., Biochim.Biophys. Acta, 79, 399 (1964).
Boman, H. and Hjertén, S., Arch.Biochem.Biophys., suppl. 1, 276 (1962).
Burton, K., Biochem. J., 62, 315, (1956).
Eagle, H., Science, 130, 432,(1959).
Hjertén, S., Biochim. Biophys. Acta, 79, 393 (1964).

Kerr, S.E. and Seraidairan, K., J.Biol. Chem., 159, 211 (1945).
Philipson, L., J. Gen. Physiol., 44, 899 (1961).
Polson, A., Biochim. Biophys. Acta, 50, 565 (1961).
Russell, B., Mead, T.H. and Polson, A., Biochim. Biophys. Acta, 86, 169, (1964).
Spirin, A.S., Macromolecular Structure of Ribonucleic Acids, Reinhold Publishing Corporation, New York, 1964.
Öberg, B., Albertsson, P.-Å. and Philipson, L., Biochim. Biophys. Acta, in press. (1965).