ADSORPTION PATTERNS OF ENTEROVIRUS RIBONUCLEIC ACID

II. CALCIUM PHOSPHATE ADSORPTION PATTERNS OF RIBONUCLEIC ACIDS OF SOME ENTEROVIRUSES

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(Received October 21st, 1965)

INTRODUCTION

Infectious RNA of type 1 poliovirus, strain Brunhilde, is eluted from calcium phosphate column in three main fractions[1]. This presents the question as to whether this is a property of the particular strain-specific RNA, or a property of RNA of all three poliovirus types, or possibly of all enteroviruses; and it would be possible to detect by column chromatography differences between the RNA of different enterovirus strains such as would correspond, for example, to differences in their antigenity or neurovirulence, *i.e.* the significant differences between enterovirus strains. The aim of the work reported here was to provide at least a partial answer to these questions.

EXPERIMENTAL

Virus

The highly neurovirulent poliovirus strains Brunhilde, MEF_1 and Saukett, and the attenuated poliovirus strains LSc, 2ab, P 712 and Leon $12a_1b$ were employed. Each group of polioviruses is type 1, 2 and 3, respectively. Coxsackie type B_1 and Echo type 8 were used as representatives of the other enteroviruses.

The preparation of viral suspensions has been described previously.

Virus concentration

The Coxsackie B_1, Echo 8 and Brunhilde strains were concentrated 10 times by means of the rapid evaporation of the water from the viral suspension in a dialysis tube. The temperature was 20°. The concentrate was dialyzed against buffered physiological saline at 4° for 16 h.

Calcium phosphate

Type 2a "old" calcium phosphate was used. The cells, solutions and media, extraction of RNA, and the chromatography are described in the previous paper[1].

RESULTS

Adsorption patterns of infectious RNA of different poliovirus strains

Type 2a "old" calcium phosphate was used to study the adsorption properties of the infectious RNA of the three neurovirulent and the three attenuated poliovirus strains. The working temperature was 20°. The RNA elution patterns for the individual strains, of differing neurovirulence, corresponded so closely that they fitted a single curve. This is demonstrated in Fig. 1, which shows that the RNA of each strain was eluted in three fractions. The ion strength of the buffer used to elute these RNA was very high.

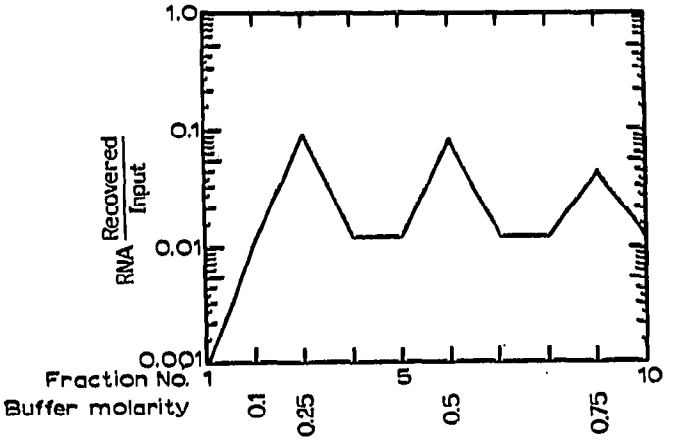

Fig. 1. Adsorption patterns of infectious RNA of different poliovirus strains.

Tables I and II present the values from which the curve in Fig. 1 was drawn. The three main fractions yielded 19.8% of the total input of RNA. On an

TABLE I

PERCENTAGE OF RNA IN THREE MAIN FRACTIONS

Molarity of the buffer	*Fraction*	*Percentage of eluted RNA*
0.25	3	8.38
0.5	6	7.54
0.75	9	3.92

TABLE II

RNA ELUTION OF SIX POLIOVIRUS STRAINS

Elution	*Percentage of eluted RNA*
Maximum	86
Minimum	2.18
Average	25.6
Elution without fractions 3, 6 and 9	6.8

average, 25.6 % of input RNA was eluted from the whole column. Hence, 6.8 % of the total RNA eluate was collected in fractions other than 3, 6 and 9. The maximum elution amounted to 86 % and the minimum to 2.18 % of RNA.

Adsorption patterns of Coxsackie B_1 and Echo 8 RNA

Coxsackie B_1 and Echo 8 viruses were selected for study as representatives of the non-poliomyelitic enteroviruses. Both viruses were concentrated 10 times. Their RNA adsorption patterns were compared with that of the poliovirus strain Brunhilde, which was also used as a tenfold concentrate. The results are shown in Fig. 2.

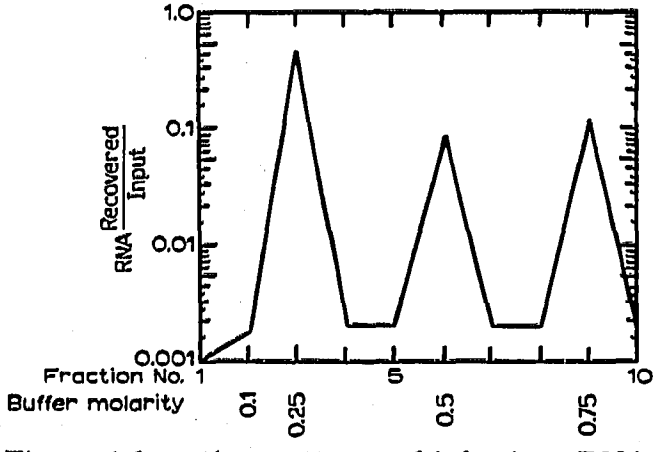

Fig. 2. Adsorption patterns of infectious RNA of different concentrated enterovirus strains.

Since the RNA of concentrated Coxsackie B_1 and Echo 8 viruses exhibited elution patterns which fully corresponded to that of the concentrated poliovirus RNA, it was again possible to fit all three elution patterns to one curve. The RNA of these

TABLE III

PERCENTAGE OF RNA IN THREE MAIN FRACTIONS

Molarity of the buffer	*Fraction*	*Percentage of eluted RNA*
0.25	3	38
0.5	6	7.1
0.75	9	11.2

TABLE IV

RNA ELUTION OF THREE ENTEROVIRUS STRAINS

Elution	*Percentage of eluted RNA*
Maximum	165
Minimum	8.2
Average	57.26
Elution without fractions 3, 6 and 9	0.96

viruses was also mainly eluted at three strong molarities of phosphate buffer, *i.e.* 0.25, 0.5 and 0.75. The numerical values from which the curve has been drawn up are given in Tables III and IV. In these three virus types, a maximum elution of 165 % and a minimum of 8.2 % of the RNA put in was obtained. Slightly less than 1 % of RNA eluate was collected in other fractions.

Irregular extraction of RNA

Infectious RNA titres were determined prior to chromatography in all the experiments. In 11 % of the cases infectious RNA could not be detected in the RNA extract. If these extracts were loaded onto the column, their eluates displayed infectious RNA. The elution pattern of this "undetectable" RNA was identical with that of RNA determined in the extract.

DISCUSSION

The adsorption properties of the RNA of both attenuated and neurovirulent strains of all three poliovirus types were studied using calcium phosphate type 2a (so-called "old"). The results indicated that the RNA of all the strains was eluted from the column in the same manner, *i.e.* in three main fractions of very high buffer molarity. The average amount of RNA eluted from the calcium phosphate column was quite low: 26.6% of total RNA input.

The correspondence of the elution patterns of all the poliovirus strains studied suggested the investigation of the adsorption patterns of other enterovirus RNA's. Coxsackie B_1 represented the Coxsackie viruses and Echo 8 the Echo viruses. Since in the non-concentrated state only small amounts of RNA were obtained with these viruses, which would have been difficult to determine in the eluate, these viruses, as well as poliovirus strain Brunhilde, were concentrated. Of the available methods of concentration, that of rapid evaporation of water from the virus suspension was selected. A tenfold concentrate which was not otherwise purified was used. This method of concentration was chosen in order to prevent the possible loss of any of the probable different components of the virus population during the procedure. The results showed that the RNA of these two non-polio viruses, as well as of the concentrated poliovirus, had the same elution pattern as all the other non-concentrated polioviruses. In this case, however, the amount of RNA eluted from the column was twice as high, *i.e.* 57 %. In contrast to the chromatography of non-concentrated poliovirus RNA, the amount of RNA eluted in the "remaining" fractions, *i.e.* other than fractions 3, 6 and 9, was substantially lower in this experiment, approximately seven times. It is difficult to explain this in a reliable and acceptable way at present. The following possible explanation of the double amount of concentrated virus RNA eluted from the column is offered.

In 11 % of the cases infectious RNA was not determined in the RNA extract from non-concentrated viruses, and was only detected after chromatographic fractionation. It can therefore be assumed that the concentrated virus RNA did not, in the unfractionated extract, manifest itself to the same degree as the RNA of non-concentrated viruses. In other words, the higher the concentration, the greater the possibility of infectious RNA blocking in the extract. Chromatography however disclosed the blocked specific infectious RNA, and the degree of this disclosure is expressed by

the difference between the eluted amount of non-concentrated and concentrated virus RNA.

The anomaly of the 11 % of cases where RNA was not detected in the extract (in so-called controls) is explained by insufficiently pure isolation of RNA; there could have been remnants of the protein envelope, so that neither pure RNA nor complete virus capable of activity in contact with cells was obtained. Chromatography itself then completely removed these protein envelope remnants.

Seeing that each virus suspension was prepared from a single plaque yield, each can be assumed to be homogeneous. Nevertheless, the RNA elution pattern gave three peaks. This would suggest that the infectious RNA of enteroviruses is not homogeneous, but consists of three distinct components in respect of adsorption properties. The analogous elution patterns of all the enteroviruses studied signify that the biological differences present among these viruses are not manifested in the calcium phosphate adsorption patterns of their RNA. However, it can be assumed that the triple fractionation of enterovirus RNA is a characteristic of the enterovirus group as a whole, in the same way as the two-peak pattern is a characteristic of foot-and-mouth disease virus RNA (comparing our results with those of BROWN *et al.*[2]).

In all these experiments, the presence of cell nucleic acids in the infectious viral RNA, and in particular their possible influence on the course of viral RNA elution, was considered. Since this influence was not noted, no attention was paid to the cell nucleic acids themselves.

SUMMARY

Infectious RNA from all strains of all three types of highly neurovirulent as well as of attenuated polioviruses presents an elution pattern of three peaks on type 2a (so-called "old") calcium phosphate. Infectious RNA of Coxsackie B_1 and Echo 8 viruses has the same adsorption pattern. Maximum elution takes place with 0.25, 0.5 and 0.75 *M* phosphate buffer.

Questions associated with enterovirus RNA fractionation are discussed.

REFERENCES

1 J. KOZA, *J. Chromatog.*, 25 (1966) 314
2 F. BROWN, J. F. E. NEWMAN AND D. L. STEWART, *Nature*, 197 (1963) 590.