SEPARATION OF DEOXYRIBONUCLEIC ACID AND RIBONUCLEIC ACID BY GEL FILTRATION

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In a current investigation in collaboration with the Microbiological Laboratories of this Institute, it was necessary to work out a method of separating ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) fractions from an extract of nucleic acids. It was important to avoid any degradation of such substances and to obtain each fraction with less than 1 % of the other component.

At first the two substances were obtained from the mixture by enzymatic hydrolysis of DNA and RNA, respectively, and removal of the hydrolysates by gel filtration on Sephadex G-50 in water. But, owing to the incomplete hydrolysis of RNA, the DNA so obtained was not free of RNA. A direct separation of the two components by gel filtration was then tried, in view of the fact that these substances, even though they have a molecular weight very much greater than those generally fractionated by gel filtration (*e.g.* proteins), are very different in structure from proteins and from each other. Sephadex G-200, a dextran of maximal porosity, was used, and to obtain the optimal experimental conditions it was tried with different buffers of increasing ionic strength and with artificial mixtures of nucleic acids.

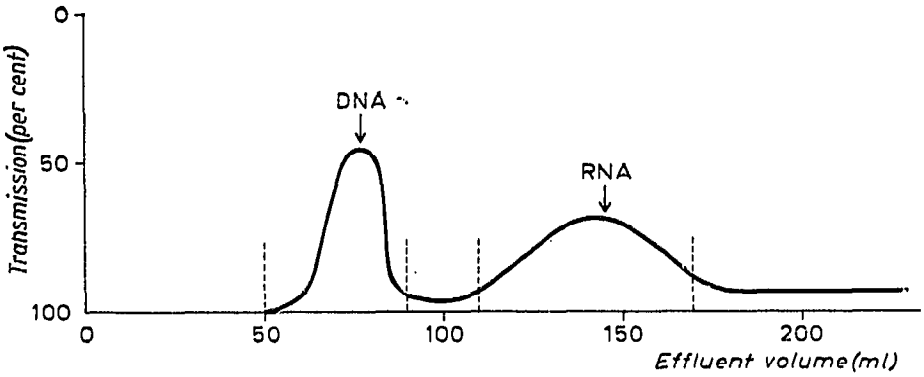

Fig. 1. Separation of DNA and RNA. Experimental conditions as reported in Table I. The vertical dotted lines limit the fractions I and II mentioned in Table II.

As a result of these tests, a complete separation of the DNA and RNA mixture into two fractions was possible as shown in Fig. 1, which illustrates the separation of a nucleic acid mixture extracted from Rhesus monkey liver by the phenol method of GIERER AND SCHRAMM[1]. The conditions are given in Table I.

The curve in Fig. 1 gives the optical transmission at 254 mμ (recorded by an LBK Uvicord ultraviolet absorptiometer) *versus* the eluate volume. Peak I is formed by DNA and the sharp peak proves that the DNA does not diffuse into the gel particles. Peak II, formed by RNA, has a long tail representing a great quantity of

TABLE I

SEPARATION OF DNA AND RNA BY GEL FILTRATION

Sample:	nucleic acids from liver, about 1 mg in 5 ml
Column:	diameter = 25 mm, height = 350 mm
Packing:	Sephadex G-200 in 0.1 *M* Tris–HCl, pH 7.2; 1 *M* NaCl
Eluent:	the same buffer as for the packing
Pressure:	700 mm of solution column
Flow:	30 ml/h
Measurement:	continuous recording of optical transmission at 254 mμ
Desalting	
Sample:	fractions I and II evaporated to 5 ml
Column:	diameter = 25 mm, height = 350 mm
Packing:	Sephadex G-50 in water
Eluent:	water
Pressure:	500 mm of water column
Flow:	500 ml/h
Measurement:	optical transmission as above and electrical conductivity

RNA of low molecular weight and oligo- and mono-ribonucleotides which were present in the extract, as shown also by chromatography on methylated albumin.

In the lower part of Table I the experimental conditions for desalting fractions I and II on Sephadex G-50 are reported, as a great quantity of salts present in the eluate must be eliminated.

In Table II the analytical results for the separation given in Fig. 1 are reported. The DNA has been determined by the diphenylamine method according to BURTON[2].

TABLE II

RESULTS OF THE CHEMICAL ANALYSIS OF A SAMPLE OF DNA AND RNA BEFORE AND AFTER SEPARATION AND DESALTING

	Volume (ml)	*DNA (μg)*	*RNA (μg)*
Sample	5	270	850
Peak I	29	264	< 0.5
Peak II	39	< 0.5	325
Peak I after desalting	—	261	—
Peak II after desalting	—	—	284

Small amounts of RNA in the DNA fraction were not determined by the orcinol method because the great quantity of DNA present interferes and gives elevated values for RNA. Therefore the following method was adopted: The samples (2 ml) are digested at 37° for one hour with an equal volume of 2 *N* NaOH. One millilitre of cooled 4 *N* $HClO_4$ is then added to the cooled solution, which is centrifuged at 4000 r.p.m. for 10 min (according to DEFRANCE *et al.*[4]). The RNA in the supernatant is determined as P according to BEREMBLUM AND CHAIN[3].

As shown in Table II the traces of RNA and DNA in fractions I and II, respectively, are near the sensitivity limits of the methods.

When the nucleic acid solution contains a DNA of low molecular weight, the RNA fraction is treated with DNAase before desalting on Sephadex G-50.

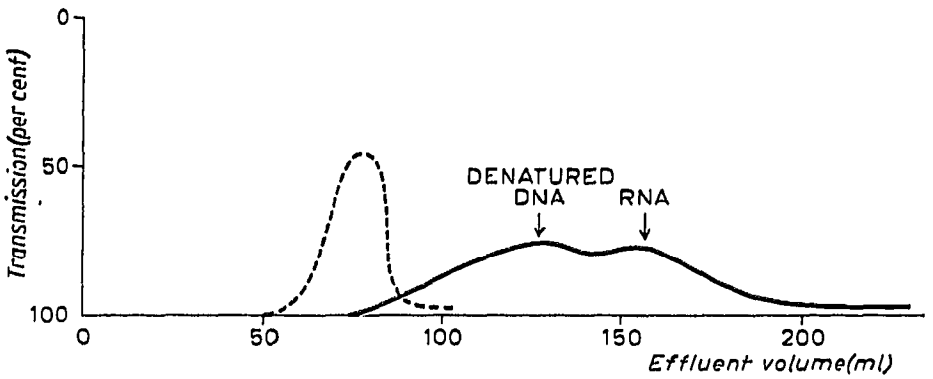

Fig. 2. Chromatogram of a sample of nucleic acids, as in Fig. 1, after alkaline denaturation. The dotted line is the DNA peak from Fig. 1.

It is probable that the separation of DNA and RNA on Sephadex G-200 is due to the difference in structure rather than the difference in molecular weight. According to this hypothesis a sample of DNA chromatographed under the conditions given in Table I after alkaline denaturation gives the elution curves shown in Fig. 2 (for comparison the non-denatured DNA is shown as a dotted line).

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SUMMARY

This paper describes the gel filtration of an extract of nucleic acids on Sephadex G-200. The experiments were performed on 2.5 × 30 cm columns with 0.1 *M* Tris–HCl pH 7.2 and 1 *M* NaCl at a flow rate of 30 ml/h. The optical transmission of the eluate was recorded.

Two peaks were obtained: peak I is due to deoxyribonucleic acid, peak II to ribonucleic acid and oligo- and mono-ribonucleotides which were present in the extract.

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