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Fractionation of Nucleic Acids by the use of Poly-L-lysine-Kieselguhr Columns

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Nucleic acids have been separated and fractionated by a wide variety of methods, the most versatile of which is column chromatography.

Benzolated DEAE-cellulose (Sedat, Kelly & Sinsheimer, 1967) I.R.C.-50a (a polycarboxylic resin, in the Mg^{2+} form), (Frankel & Crompton, 1962), hydroxyapatite (Bernardi, 1965) and methylated albumin-coated kieselguhr (Mandell & Hershey, 1960) are among the methods that have been used to try to obtain some degree of resolution between DNA and RNA and to differentiate between DNA molecules differing in some physical or chemical property.

Ayad & Blamire (1968) showed that elution of the DNA of *Bacillus subtilis* strain Marburg from poly-L-lysine kieselguhr (PLK) columns with a linear gradient of increasing salt concentration gave an elution profile that, when continuously monitored at 257m μ , contained three distinct peaks.

In the present study PLK fractionation, which appears to depend mainly on the base composition of the nucleic acids and to a lesser extent on the secondary structure, was used to separate mixtures of RNA and DNA prepared by the method of Marmur (1961) from *B. subtilis* strain Marburg. The RNA peak appeared as a separate component, at 1.0–1.1 M-NaCl, well separated from the main DNA peak, at 2.0 M-NaCl, and was identified by assaying the fractions obtained from a standard PLK elution by using the orcinol assay for RNA (Albaum & Umbreit, 1948) and the diphenylamine assay for DNA (Burton, 1956). Treatment of the mixture, or fractions, with ribonuclease removes this peak, leaving only the standard DNA elution profile.

The region between the RNA and DNA peaks is being investigated and appears from the initial results to contain 'protected' DNA. The form this protection takes is not certain, but RNA and protein are both present and the amount of each can be decreased by extensive deproteinization with agents such as chloroform-3-methylbutan-1-ol, followed by further ribonuclease treatment.

The main DNA peak, whose elution commences at 2.0 M-NaCl, was assayed for transforming activity by using *B. subtilis* strain 31, which is auxotrophic for histidine and tryptophan. It was found that when individual fractions across this peak, which are successively higher in guanine-cytosine content, were assayed for both markers the histidine gene was found to be eluted earlier than the tryptophan gene. Thus the results suggest that at least a partial separation has been achieved of DNA corresponding to the genes associated with the synthesis of histidine and tryptophan.

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Quenching of Fluorescence of Deoxyribonucleic Acid-Bound Hydrocarbons

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When a polycyclic hydrocarbon such as benzo[a]pyrene is solubilized in aqueous DNA solution, the fluorescence maxima are shifted to longer wavelengths compared with those in free solution and the overall intensity is considerably decreased (Boyland & Green, 1962, 1964; Liquori, De Lerma, Ascoli, Botré & Trasciatti, 1962).

Under conditions favouring hydrocarbon solubilization (i.e. with DNA dissolved in glass-distilled water) the intensity of benzo[a]pyrene or perylene fluorescence in such solutions consistently decreases as the pH is lowered from 7.5 to 5, though the extent of the quenching varies (generally three- to seven-fold). The ultraviolet-absorption spectrum of the DNA indicates that cytosine residues are starting to be titrated over this pH range under these conditions of low ionic strength.

Addition of Ag^+ ions to such DNA-benzo[a]pyrene solutions (Green, 1967) at a silver nitrate/DNA phosphate ratio <0.15 causes a similar quenching of hydrocarbon fluorescence. The