Chromatographic fractionation of the transforming principle of the pneumococcus*

Studies on the genetic transformations of bacteria by purified deoxyribonucleate which have been carried out hitherto have utilized specimens from the entire DNA pool extracted from the donor cells. Presumably only a small part of this material is active in the transformation of any single character. The present paper demonstrates a chromatographic technique, in principle similar to that employed by BROWN AND WATSON[1] to provide an apparent fractionation of DNA, by means of which pneumococcal DNA may be resolved into reproducible fractions which exhibit pronounced differences in their activity for transformation to streptomycin resistance.

The donor strain of streptomycin-resistant pneumococcus is grown in the presence of radioactive phosphate in a complex medium (Difco heart infusion-tryptose) which has initially been freed of phosphate by passage at pH 2 through a column of Amberlite IR-45 (acetate). The harvested cells are lysed, precipitated with alcohol, redispersed in saline, and deproteinized by treatment with sodium lauryl sulfate and shaking with chloroform. The final preparations consisted of from seventy-five to ninety-seven percent DNA, the remainder being largely RNA.

Chromatography is carried out by gradient elution on a column consisting of methylated bovine serum albumin[2] mixed with twenty parts of Celite. DNA is applied in a small volume of 0.1 *M* saline, buffered near neutrality, and is quantitatively bound at the top of the column. The material is then washed with a buffered solution of an appropriate salt (*e.g.*, 0.10 *M* NaCl) below its eluting concentration. The fluid which continues to percolate through the column is gradually fortified with a slowly rising concentration of the same salt or a second salt. Where two different salts have been employed, their proportions have been regulated so as to maintain a constant total ionic strength. A continuous recording of the radioactivity of the effluent fluid provides a detailed chromatographic pattern of the eluted DNA. The effluent is collected in successive half-ml fractions, which are precipitated with ethyl alcohol and re-dissolved in sterile saline for assay of transforming activity.

When only sufficient DNA is added to a competent culture of 10^7 to 10^8 cells/ml to transform at most 10^3 cells/ml to streptomycin resistance, the number of cells actually transformed under the conditions employed is proportional to the amount of DNA added. The number of cells transformed per unit DNA, as measured by the ^{32}P activity, is taken as an index of the specific transforming activity of each fraction. For the assay, 0.1 ml of each fraction, after suitable dilution, is added to one ml of a culture of sensitive cells which have been grown four to five hours in the presence of 0.2% bovine serum albumin and chilled to 23° for fifteen minutes[3]. After ten minutes incubation at 37°, the cultures are chilled in ice and an aliquot plated in soft blood agar. The plates are incubated at least an hour and then overlaid with three ml of soft agar containing two mg streptomycin. Dark colonies surrounded by distinct halos originate from each of the transformed cells, and may easily be counted after twenty hours incubation. No such colonies are found in the absence of transforming DNA when as many as 10^8 cells are plated.

A survey of the eluting properties of several salts revealed considerable variation in the minimum concentration necessary to initiate elution, depending on the nature of both anion and cation. The anions form a typical Hofmeister series, such as is found for their binding to proteins; the cation effects, however, are much larger than those seen with proteins[4]. The quaternary ammonium salt, choline chloride, even at a concentration of 3 *M*, fails to dissociate the DNA-protein complex to any significant extent, while dissociation by sodium chloride is nearly complete below 1 *M*. It is possible, therefore, to elute the DNA at a constant ionic strength, using a gradient in which either the anion or the cation remains constant, while a cation or an anion which is relatively ineffective for elution is exchanged for an effective one. Chromatography has been carried out in both ways by pairing choline perchlorate with sodium perchlorate and pairing sodium cacodylate with sodium perchlorate. The data permit the conclusion that the formation of a complex between DNA and the basic protein displaces cations and anions, respectively, bound to each, and that elution is determined not by the ionic strength of the medium but by the competition of its ions for the interacting charged sites of both the DNA and protein.

A typical chromatogram obtained by simple elution with a sodium perchlorate gradient (buffered at pH 6.7 with 0.01 *M* phosphate) is shown in Fig. 1A. At 24.0 ml the perchlorate concentration is 0.168 *M*, and is increasing continuously by 0.011 *M* with each subsequent ml. Similar results are obtained by the other elution techniques. Some variability in pattern is found among different DNA preparations. Fig. 1B shows the result of re-application to the same column of the DNA collected within the shaded zone of 1A. Similarly, Fig. 1C shows the repetition of chromatography of DNA from the shaded zone of 1B. These figures demonstrate the actual segregation of original DNA into more homogeneous components which retain their chromatographic identity throughout repeated fractionations.

* Aided by a research contract with the Division of Biology and Medicine of the Atomic Energy Commission and an institutional grant from the Damon Runyon Fund.

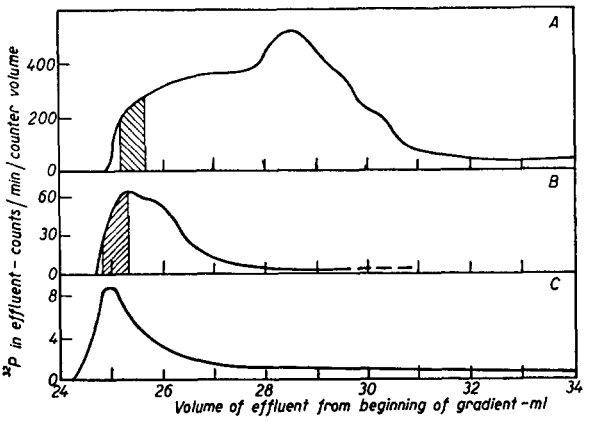

Fig. 1. The chromatography of DNA by gradient elution with sodium perchlorate (containing 0.01 *M.* phosphate buffer, pH 6.7). A: Crude DNA from pneumococcus; B: Repeated chromatography of the fraction collected within the shaded zone of chromatogram A; C: Repeated chromatography of the fraction collected within the shaded zone of chromatogram B. The salt concentrations of fractions corresponding to the same volume of effluent in A, B, and C are equal within an error of less than difference between successive fractions.

Data comparing the transforming activity and the DNA content as measured by the ^{32}P activity of selected fractions from the experiments shown in Figs. 1 A and 1 B are given in Tables I A and I B. Corresponding data for another chromatogram of somewhat differently prepared material are given in Table II. These figures demonstrate that the specific transforming activity of the best fraction obtained is twice that of the original unfractionated material, and the ratio of activities of the most and least active fractions is at least ten, and in some experiments, about fifteen. Although only a rough computation of the total recovery of transforming activity can be made, it indicates no detectable loss.

While it is evident that a pronounced fractionation of transforming activity has been achieved, together with some degree of purification of the most active DNA, these experiments do not elucidate the basis of the separation. It has been shown[4,5] that the salt concentration required to dissociate DNA from histone complexes varies as the ratio of the sums of the adenine + thymine content

TABLE I

FRACTIONATION OF PNEUMOCOCCAL DNA: REPEATED CHROMATOGRAPHY

A: First passage through column (chromatogram shown in Fig. 1a)

					Unfrac.
a. *Fraction number*	26	29	32	36	DNA
b. *Cells transformed* by a standard dilution of each fraction	110	317	265	62	226
c. *DNA content*	145	861	1527	444	505
d. *Specific transforming activity*	3.03	1.46	0.69	0.56	1.42

B: Second passage through column; rechromatography of fraction 27 from the set given above (chromatogram shown in Fig. 1b)

				Unfrac.
a. *Fraction number*	36	38	40	DNA
b. *Cells transformed* by a standard dilution of each fraction	320	91	19.4	940
c. *DNA content*	108.2	92.0	27.5	563
d. *Specific transforming activity*	2.96	0.98	0.77	1.66

Fraction number designates the position of the fractions in the sequence of 0.5 ml fractions collected, numbered from an arbitrary starting point.

Cells transformed is the number of colonies formed in the presence of streptomycin from a 0.2 ml aliquot of a 1 ml culture to which had been added 0.1 ml of appropriately diluted DNA.

DNA content is given by the amount of ^{32}P in each fraction in counts/sec/ml and the amount of DNA ^{32}P in the unfractionated material.

Specific transforming activity is the number of cells transformed per unit DNA.

TABLE II

FRACTIONATION OF PNEUMOCOCCAL DNA

Fraction number	31	33	36	38	*Unfract. DNA*
a. *Cells transformed* by 10^{-2} dilution	—	489	86	61	
b. *Cells transformed* by 10^{-3} dilution	154	51	8	4	163
c. *DNA content*	17.6	10.6	7.53	7.40	29.9
d. *Specific transforming activity*	43.7	24.0	5.72	4.13	27.3

to that of guanine + cytosine. In view of the close relationship between the chromatographic and the simple dissociation processes, it may be presumed that the same change in ratio of the bases obtains from the front to the rear of the chromatographic zone. Nevertheless, the evidence is insufficient to favor the simple hypothesis that each genetic unit of DNA reaches a characteristic chromatographic position because of a particular and characteristic ratio of bases in its molecule. It is also possible that the position and base ratio reflects: differences in some as yet uncharacterized state of various types of DNA; differences in degree of degradation; differences in extent of irreversible association among the genetic units (varying lengths of chromosome segments). Experiments designed to clarify some of these possibilities are in progress.

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Received May 26th, 1955

* Contribution No. 32.

The biosynthesis of methionine from homocysteine and methylmethionine sulfonium salt*

The microbiological activity of methylmethionine sulfonium salt (MMS) has been reported by MCRORIE *et al.*[1]. It was found that this compound can replace the methionine requirement of some strains of methionine auxotrophs of *E. coli*. Since the sulfonium derivative was as much as three times as effective as methionine in supporting growth of these mutants, it was suggested that MMS serves a role more complex than simple conversion to methionine. SCHLENK AND DEPALMA[2] have shown that MMS in combination with homocysteine supports greater production of methylthioadenosine in *Torulopsis utilis* than could be accounted for by simple conversion to methionine. It seemed of interest, therefore, to test the ability of MMS and homocysteine to replace the methionine requirement of auxotrophs of *Aerobacter aerogenes* which can utilize methylthioadenosine in the biosynthesis of methionine[3,4]. Methylmethionine sulfonium iodide was prepared according to the procedure of TOENNIES AND KOLB[5]. All other compounds used were commercial products. Both homocysteine and MMS were sterilized by filtration.

A summary of typical results is presented in Table I. It can be seen that MMS alone would not support growth of either methionine auxotroph 62 or 68. Homocysteine permitted less than half maximal growth of both cultures. However, the combination of MMS and homocysteine permitted growth nearly equal to that of equimolar concentrations of methionine. It is interesting that both cultures responded well to the two compounds although only auxotroph 68 can utilize methylthioadenosine as well as methionine. Prolonged incubation up to 96 hours or the use of very heavy inocula did not affect the pattern of results. No growth occurred in the presence of cysteine and MMS. This is in contrast to the experiments of STEKOL[6] who found that this combination would permit growth of rats on a methionine-deficient ration.

* Work performed under the auspices of the U.S. Atomic Energy Commission.