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FRACTIONATION OF MAMMALIAN DNA ON DEAE-CELLULOSE

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SUMMARY

Chromatography on a DEAE-cellulose (DE-52) column of native and denatured DNA from P388F cells has been studied. The main bulk of native DNA is eluted at 0.8 *M* NaCl and a minor fraction is eluted with 0.5 *N* NaOH. The proportion of the DNA components obtained depends on the type of isotopic labelling used and the method of storing of the DNA preparation following isolation. Heat-denatured DNA elutes in 2 *M* NaCl mainly within the pH gradient from 0.1–1.0 *M* NH_4OH.

When native DNA is chromatographed on DEAE-cellulose, a small fraction of the DNA elutes at 0.5 *N* NaOH. This fraction is double-stranded, as determined by hydroxyapatite chromatography. A similar component predominates, however, when "newly synthesised" DNA is fractionated. The profiles obtained with "newly synthesised" and "template" labelled DNA differ in their undenatured and heat-denatured configurations. The presence of formaldehyde during the chromatography of undenatured DNA leads to an increased homogeneity of the profile and during heat denaturation considerable modifications to the profile are observed. Some of the changes can be explained in terms of a decrease in the heterogeneity of the charge distribution on the DNA. The technique appears to combine a high degree of reproducibility with sensitivity to charge clustering along the DNA.

INTRODUCTION

Fractionation of DNA in the 20–30S range of molecular weights has been undertaken by a wide variety of procedures. Of the column chromatographic procedures that have been described, methylated albumin kieselguhr[1], hydroxyapatite[2–4] polylysine-coated kieselguhr[5], DEAE-cellulose (DE-52)[6], ECTEOLA-cellulose[7] and benzoylated naphthoylated DEAE-cellulose[8,9] have been most successful. Although kieselguhr and hydroxyapatite systems give clear fractionation of single, double and hybrid structures, the anion exchangers, in particular DEAE-cellulose and its derivatives, give a complex spectrum of peaks which are not yet fully characterised[7,10].

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More detailed studies with the ion-exchange celluloses have revealed marked differences in profile between DNAs isolated from different tissues[11] of the same animal, but this has not been confirmed by other workers[12]. However, different methods of preparation of the DNA from the same tissue are also known to produce different chromatographic profiles[13–15].

Kit[13] undertook a broad comparative study of both ECTEOLA-cellulose and DEAE-cellulose anion exchangers and found that with DEAE the fractions were eluted at higher molarities of salt and higher pH values. With both exchangers, one DNA was eluted in 0.6 *M* NaCl and several others in 2 *M* NaCl within the pH gradient from 0.1–1.0 *M* ammonium hydroxide. The fraction of the DNA eluted in 0.6 *M* NaCl was found to increase if the DNA was heated at 100° for 15 min or if there was DNAase activity during the preparation of the tissue.

DEAE-cellulose columns have, up until now, been mainly used for chromatography of RNA and DNAase digests of DNA[16–18], whereas the fractionation of DNA on DEAE-cellulose paper strips or centrifuged paper pulp has been more extensively used[6]. The conclusions derived from these methods were that fractions eluted at high pH values and high salt concentrations tended to be more polymerised. Preliminary experiments[19] indicated that the technique of fractionation of mammalian DNA on columns of DE-52 showed considerable sensitivity and reproducibility. The main bulk of native DNA can be shown to be eluted with 0.75 *M* NaCl at neutral pH, whereas denatured DNA was eluted mainly in 2 *M* NaCl within the pH gradient from 0.1–1.0 *M* ammonium hydroxide. The main peak eluting at 0.72 *M* NaCl was double stranded but the fraction eluting at strong alkaline pH could be either single or double stranded in the original sample.

The work presented here reports the DE-52 chromatographic analysis of mammalian DNA obtained from native and denatured "newly synthesised" and "template" DNA from P388F cells labelled with both [^{3}H]- and [^{14}C]-thymidine.

EXPERIMENTAL

Cells and DNA labelling

The cell line used was the murine lymphoma line P388F. The origins and conditions for liquid suspension culture have been described previously[20]. Two methods of cell labelling were employed. For "newly synthesised" DNA, a 1-litre suspension of P388F cells in logarithmic phase growth was incubated in Fischer's medium, supplemented with 10% horse serum at 37°, and 0.2 μCi/ml of [^{3}H]thymidine (25 Ci/mmole) was added for a period of 30 min. The cells were then harvested and centrifuged for 15 min at 4° and 1200 × *g*. The supernatant was then removed, and the pellet of cells washed twice with isotonic saline. The supernatant was discarded and the DNA extracted from the cells as described below.

For "template" DNA labelling, the procedure was essentially as for "newly synthesized" DNA, except that [^{3}H]thymidine (0.2 μCi/ml; 25 Ci/mmole) was added for 6 h. The cells were then centrifuged as before, fresh medium was added, and the incubation continued for a further 18 h with no labelled precursors present, to allow the cell number to double, before extraction of the DNA.

In later experiments, where specified, labelling was carried out using 1 μCi/ml of [^{3}H]thymidine (5 Ci/mmole) or 0.05 μCi/ml of [2-^{14}C]thymidine (54.5 mCi/mmole).

DNA preparation

The DNA was extracted by a phenol extraction procedure[21]. 40 ml 6% *p*-aminosalicylate (PAS) were added to the washed cell pellet and homogenised gently with a glass rod to cause lysis of the cells. An equal volume of a phenol–hydroxyquinoline mixture (88:0.1) was added and the whole shaken for 1 h at room temperature. After centrifugation at 1800 × *g* for 1 h at 5° the upper PAS layer was carefully removed. Interfacial material if present was re-extracted with half the original volume of PAS. The upper PAS layers were pooled, cooled in ice, and an equal volume of cooled 2-ethoxyethanol was added and gently mixed. The DNA was spooled out of the solution and dissociated into water (70% of the volume of the original PAS used) containing 2.5 mg sodium acetate. Approximately 0.2 mg pancreatic ribonuclease was added and the mixture was left to stand overnight at 2°. The RNAase digest was made 4% with respect to sodium acetate and deproteinised with an equal volume of chloroform–*n*-butyl alcohol (3:1) by shaking for 15 min at room temperature. After centrifugation for 15 min at 5° and 1500 × *g*, the aqueous phase was carefully removed, cooled in ice and the DNA collected on a glass rod following addition of an equal volume of 2-ethoxyethanol. The DNA was transferred to 70% ethanol containing 2% sodium acetate for 10 min in the cold, and then to 95% ethanol for 10 min. The DNA was unrolled from the rod with a spatula into the 95% ethanol.

In the initial experiments the DNA was dried by the addition of absolute ethanol followed by absolute ethanol–dry ether (1:1) and finally calcium chloride-dried ether. Following removal of the ether, the DNA was placed in a vacuum desiccator for 20 min. DNA that was stored in this dry state did not dissolve in dilute 0.01 *M* phosphate buffer of pH 7.0.

In later experiments, where specified, the DNA was not taken to dryness, but its isolation terminated at the 95% ethanol stage. The DNA was then allowed to dissociate in 0.001 *M* phosphate buffer, after which sodium chloride was added to the solution to give a final concentration of 1 *M*, in an attempt to protect against any denaturation during storage.

Heat denaturation of DNA

DNA at various concentrations (15–150 μg/ml) was denatured in a 0.1 *M* NaCl/0.01 *M* phosphate buffer of pH 6.8 by heating for 40 min in a boiling water-bath, and then rapidly chilled in an ice-bath. In some specified cases, 1% formaldehyde was also present during denaturation.

Pancreatic deoxyribonuclease I (DNAase) digestion

0.07 ml of a DNAase solution (100 μg/ml in a 0.005 *M* $MgCl_2$/0.01 *M* phosphate buffer of pH 7) was added to 3 ml of a DNA solution (15 μg/ml in a 0.1 *M* NaCl/0.01 *M* phosphate buffer of pH 6.8) and incubated at 35° for 1 h, after which time an increase in absorbance at 260 nm of 26% had occurred. The ratio of DNA to DNAase was 6.43. The enzyme reaction was terminated by addition of an equal volume of 5% EDTA in 0.3 *M* sodium dodecyl sulphate and chilling.

Preparation of T4 phage DNA

The DNA was isolated from purified virus particles by a water-saturated phenol extraction procedure described by Ritchie and Malcolm[22].

DEAE-cellulose chromatography

Following removal of fines a slurry of pre-swollen DE-52 (Whatman) equilibrated in the first eluting buffer, *i.e.* either 0.01 *M* phosphate buffer of pH 7.0 or 0.1 *M* NaCl/0.01 *M* phosphate buffer of pH 6.8, was poured into a 30 cm × 1.6 cm column and allowed to settle under gravity. The column was eluted with the same buffer (25 ml/h) until the bed height was constant at about 10–12 cm, which took 1–2 h.

Samples of DNA (up to 0.5 mg) were applied to the column, at concentrations of 15–150 μg/ml, in either 0.01 *M* phosphate buffer of pH 7.0 or 0.1 *M* NaCl/0.01 *M* phosphate buffer of pH 6.8, depending on the elution procedure.

The eluting buffers used were based on those described previously[7] for use on ECTEOLA-cellulose. However, linear gradients were used throughout instead of the stepwise elution adopted by these workers to avoid artifacts due to sudden changes in solvent composition. The gradient sequence is illustrated in Table I.

In later experiments, the initial 200 ml phosphate buffer was omitted. The elution procedure took approximately 60 h to complete, the flow-rate being approximately 35 ml/h. The gradients were run continuously with little or no interruption. The sodium ion was monitored using a sodium ion responsive glass electrode mounted in a micro flow-through cell (Type SMF, Electronic Instruments) and UV absorption of the effluent was continuously monitored using an LKB UV monitor, 10-ml fractions were collected.

Radioactivity was assayed by mixing 3-ml aliquots in 7 ml of scintillant mixture [8 g 2,5-diphenyloxazole (PPO) and 0.2 g 1,4-bis-(5-phenyloxazolyl-2)-benzene (POPOP) in 300 ml toluene and 700 ml Triton X-100][23]. Alkaline fractions were neutralised with hydrochloric acid before addition to the scintillant.

Certain fractions were dialysed against 0.01 *M* phosphate buffer of pH 7 to remove salt, followed by lyophilisation and redissolution of the DNA in the appropriate buffer required by the technique being used.

TABLE IA

ELUTING SOLUTION COMPOSITION

Solution	*Composition*
1	0.01 *M* phosphate buffer, pH 7.0
2	0.5 *M* NaCl/0.1 *M* phosphate buffer, pH 6.35
3	2.0 *M* NaCl/0.01 *M* phosphate buffer, pH 5.95
4	2.0 *M* NaCl/0.01 *M* phosphate/0.1 *M* NH_4OH, pH 10.0
5	2.0 *M* NaCl/0.01 *M* phosphate/1.0 *M* NH_4OH, pH 11.0
6	2.5 *N* NaOH, pH 13.0

TABLE IB

ELUTION SCHEME

Region	*Gradient elution scheme**	*Approximate number of fractions*	
A	200 ml (1)	0– 20	
B	20 ml (1) to 200 ml (2)	21– 60	linear gradients
C	200 ml (2) to 200 ml (3)	61–100	
D	200 ml (3) to 200 ml (4)	101–140	
E	200 ml (4) to 200 ml (5)	141–180	
F	200 ml (6)	181–200	

* The figures in parentheses refer to the solutions listed in Table IA.

Hydroxyapatite chromatography

Hydroxyapatite (Bio-Gel HTP) chromatography was used according to the method of Bernardi[3,4]. After removal of the fines, a column was prepared (5–6 cm × 1.3 cm diameter) and allowed to settle under gravity. The column was then equilibrated with the starting buffer, 0.1 *M* potassium phosphate in 3 *M* KCl, prior to elution of the DNA (0.3–20 μg/ml in 0.1 *M* potassium phosphate buffer in 3 *M* KCl, pH 6.8) with potassium phosphate buffer. A linear molarity gradient was produced by mixing 100 ml of 0.1 *M* potassium phosphate buffer in 3 *M* KCl and 100 ml of 0.4 *M* potassium phosphate buffer in 3 *M* KCl (flow-rate approximately 20 ml/h) and the column was maintained at 21–22°. Calf thymus DNA (20 μg in 0.2 ml) was added to each fraction (3 ml) followed by 3 ml of 10% trichloroacetic acid. The samples were retained at 4° overnight and the precipitate of DNA was filtered onto glass-fibre discs (Whatman GF/C), washed with ice-cold 2.5% trichloroacetic acid, dried under an IR lamp, and assayed in 4 ml of a toluene solution of PPO (0.4%) and POPOP (0.01%).

Alkaline sucrose gradient centrifugation

A linear gradient was prepared using 6.8 ml of 20% and 7.2 ml of 5% sucrose

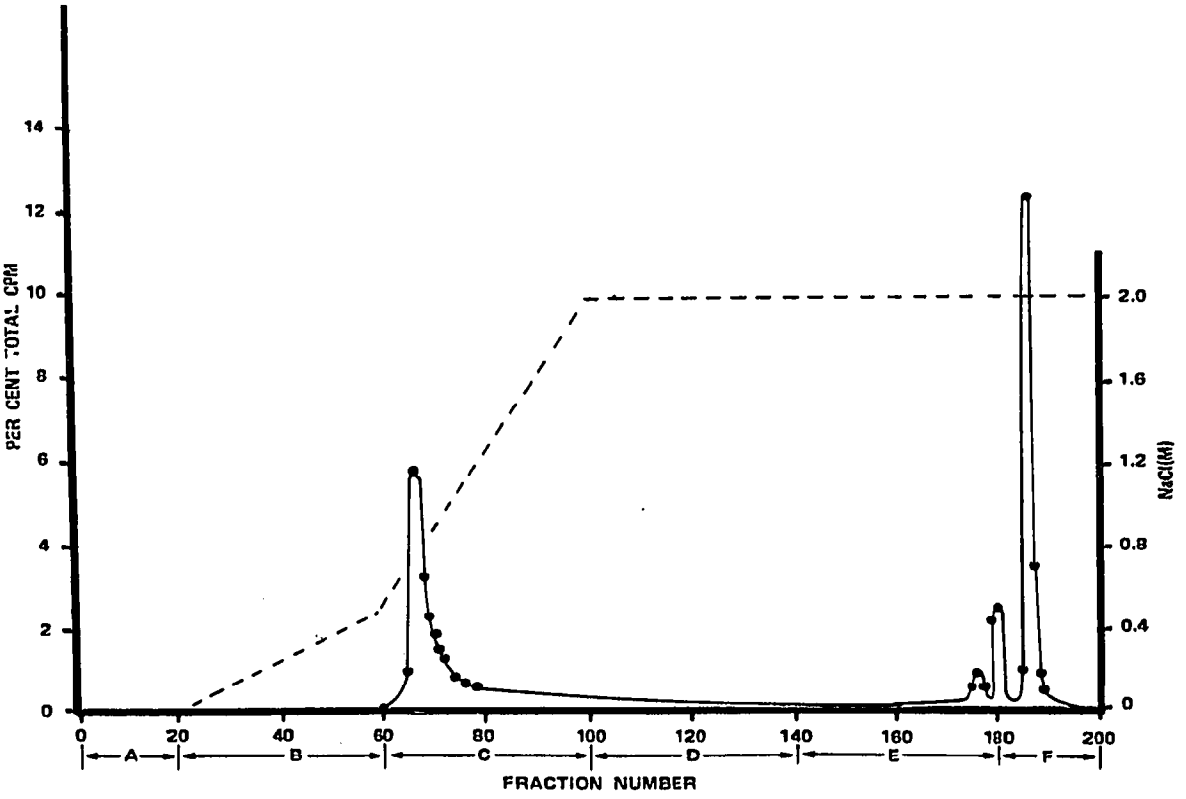

Fig. 1. Chromatography on DE-52 of non-dried ^{3}H-labelled "template" P388F DNA (465 μg). Specific activity, 6.3 μCi/mg. The salt gradient is indicated as a dashed line. Total cpm, 1.71×10^5.

TABLE II
ELUTION PROFILES OF RADIOACTIVITY

Experimental details	*% Radioactivity per gradient*					
	A	*B*	*C*	*D*	*E*	*F*
^{3}H-Labelled "template" DNA preparation, dried. Specific activity, 2730 nCi/mg	0.06	0.15	10.62	11.76	27.42	49.98
^{3}H-Labelled "template" DNA preparation, dissolved in 1 *M* NaCl. Specific activity, 6310 nCi/mg	0.33	0.93	34.90	20.17	23.91	19.75
^{3}H-Labelled "template" DNA preparation, dissolved in 1 *M* NaCl. Specific activity, 67,400 nCi/mg. 200 ml 0.01 *M* phosphate buffer (A) omitted from the elution procedure	—	0.07	40.60	15.67	12.93	30.72

containing 0.9 *N* NaOH, 0.001 *M* EDTA, 0.1 *M* NaCl and 0.01 *M* Tris of pH 12.5. Centrifugation was carried out for 5 h at 21° and 76,700 × *g* (MSE 65 Mk II centrifuge, utilising the 6 × 15 ml swing-out rotor). The gradients were fractionated into 12-drop fractions, which were diluted and neutralised with 4.4 ml of 0.125 *N* HCl. Each

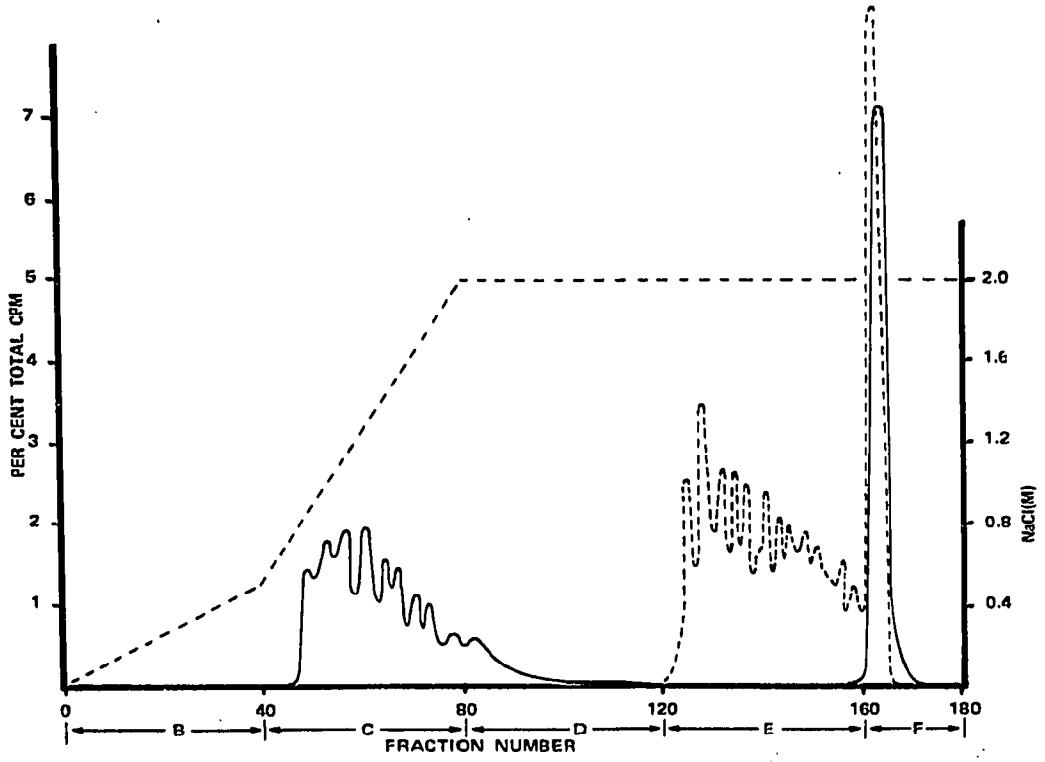

Fig. 2. Chromatography on DE-52 of non-dried ^{3}H-labelled "template" P388F DNA (450 μg) Specific activity, 67.4 μCi/mg. ——, Native DNA; - - - -, heat-denatured DNA. The salt gradient is indicated as in Fig. 1. Total cpm, 6.86 × 10^6.

fraction was then assayed in 5 ml scintillator containing 6 g PPO and 0.15 g POPOP per litre in a mixture of 400 ml toluene and 600 ml Triton X-100. DNA samples were applied to the gradients in 0.01 *M* phosphate buffer of pH 7, together with a suspension of T4 phage, also in phosphate buffer, as reference.

RESULTS

DEAE-cellulose chromatography of "template" ^{14}C-labelled DNA and the effect of initial drying

Two main peaks were observed (Fig. 1) when native DNA was applied to the column. The DNA used in this fractionation was not taken to dryness, but dissolved in 0.001 *M* phosphate buffer of pH 7.0, and subsequently made 1 *M* with respect to NaCl. 34.9% of the total radioactivity was eluted in the second gradient with 0.8 *M* NaCl (region C) and 19.7% with 0.5 *N* NaOH (region F). Hydroxyapatite chromatography indicates that the region C material is the main bulk of the double-stranded DNA (Fig. 8) whereas the region F radioactivity is more complex and consists of components which strongly adhere to the column (Fig. 9). The remaining DNA is eluted at a low level throughout the other gradients. The effect of drying the DNA on the elution profile is to reduce the amount eluting at 0.8 *M* NaCl to a considerable

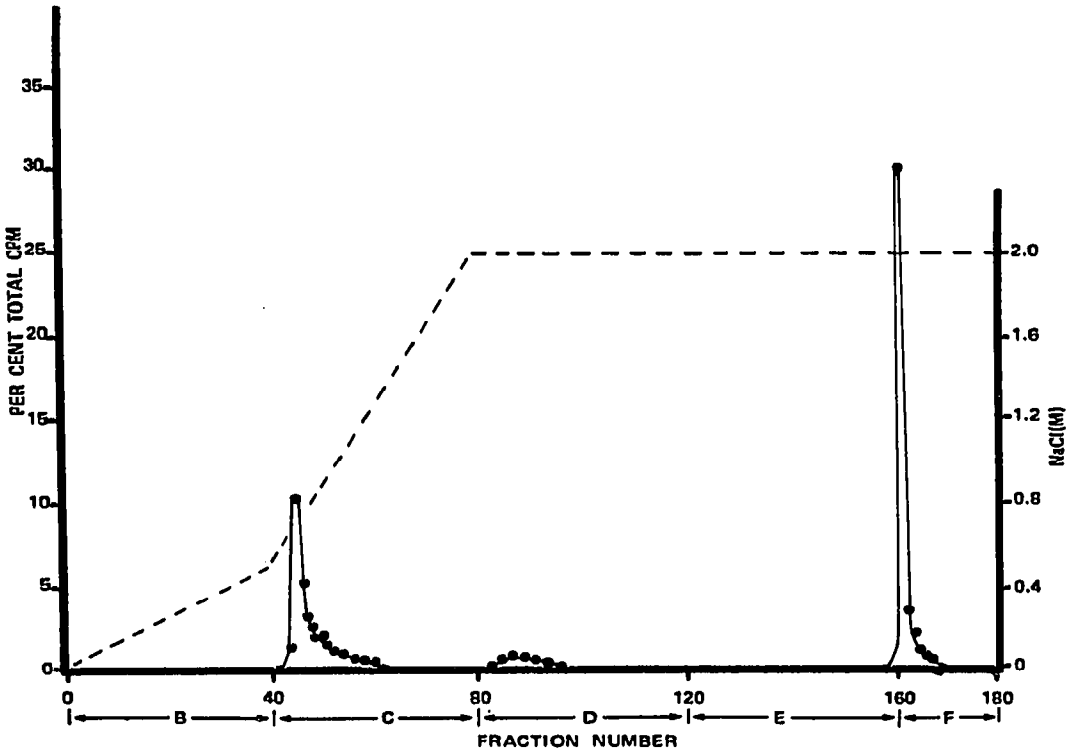

Fig. 3. Chromatography on DE-52 of non-dried ^{3}H-labelled "template" P388F DNA (34 μg) treated with 1% formaldehyde prior to application to the column. Specific activity, 67.4 μCi/mg. The salt gradient is indicated as in Fig .1. Total cpm, 4.83×10^{5}.

extent (35% in non-dried DNA to 10.6% in dried DNA). A corresponding increase in the DNA eluting with 0.5 *N* NaOH was observed from 19.7 to 49.9%. This suggests that the damage to the DNA produced by initial drying results in a modified DNA with stronger adherence to the column[24], presumably by an increased negative-charge density along the strand.

Omission of the initial 200 ml phosphate buffer from the elution schedule was found to produce a slight increase in region C radioactivity from 34.9 to 40.6% (Table II). In this case 30.7% of the initial total DNA label was eluted with 0.5 *N* NaOH (region F) and relatively less within the intermediate gradients (Fig. 2), also the main peak around 0.8 *M* NaCl was less sharp. Both these effects may be attributed to damage to the DNA by the very high specific activity of the DNA used. Application of smaller amounts of the same high-specific-activity DNA, however, resulted in the same relative amounts of label in regions C and F but a sharpening of the peak in region C occurred.

On heat denaturation of the DNA, a completely different elution profile is obtained (Fig. 2). The peak in region C, at 0.8 *M* NaCl, is now absent and more radioactivity appears at more alkaline pH and higher salt molarity; 70% is present in region E (to 1.0 *M* NH_4OH) and 25% in region F. This further suggests that the main peak at 0.8 *M* NaCl is double stranded, whereas the DNA eluted at high pH and salt

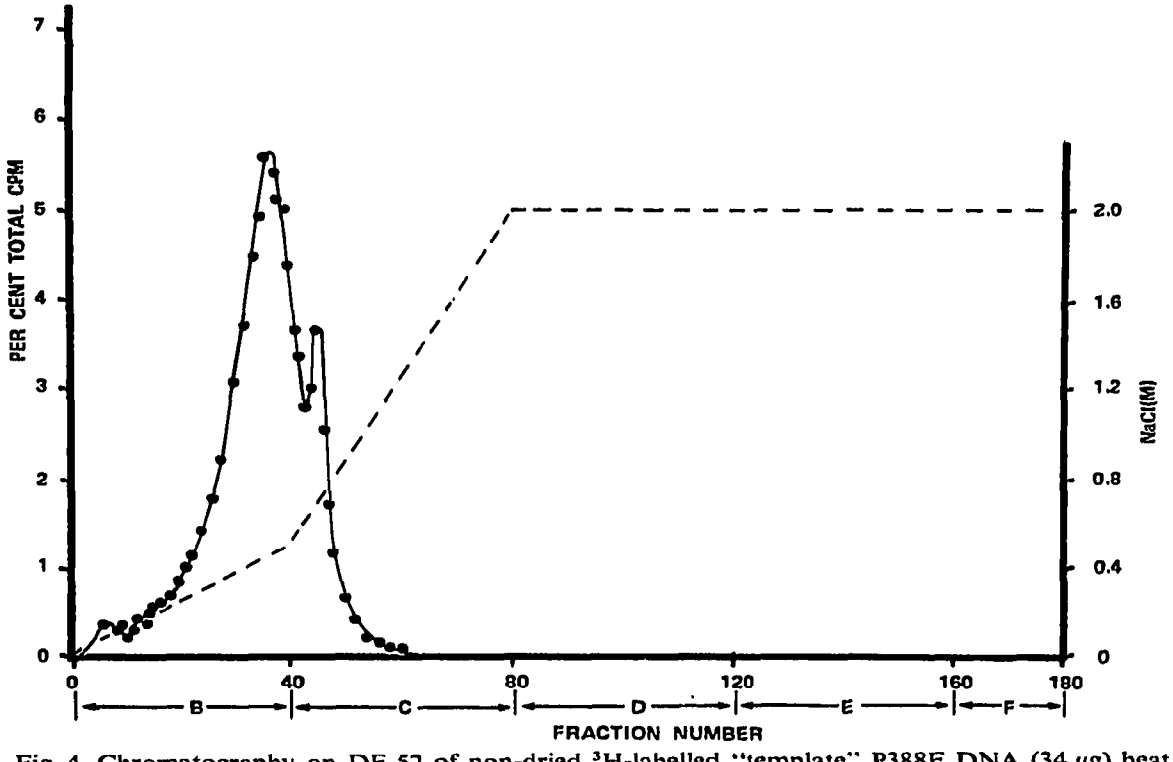

Fig. 4. Chromatography on DE-52 of non-dried ^3H-labelled "template" P388F DNA (34 μg) heat denatured in the presence of 1% formaldehyde. Specific activity, 67.4 μCi/mg. The salt gradient is indicated as in Fig. 1. Total cpm, 5.05×10^5.

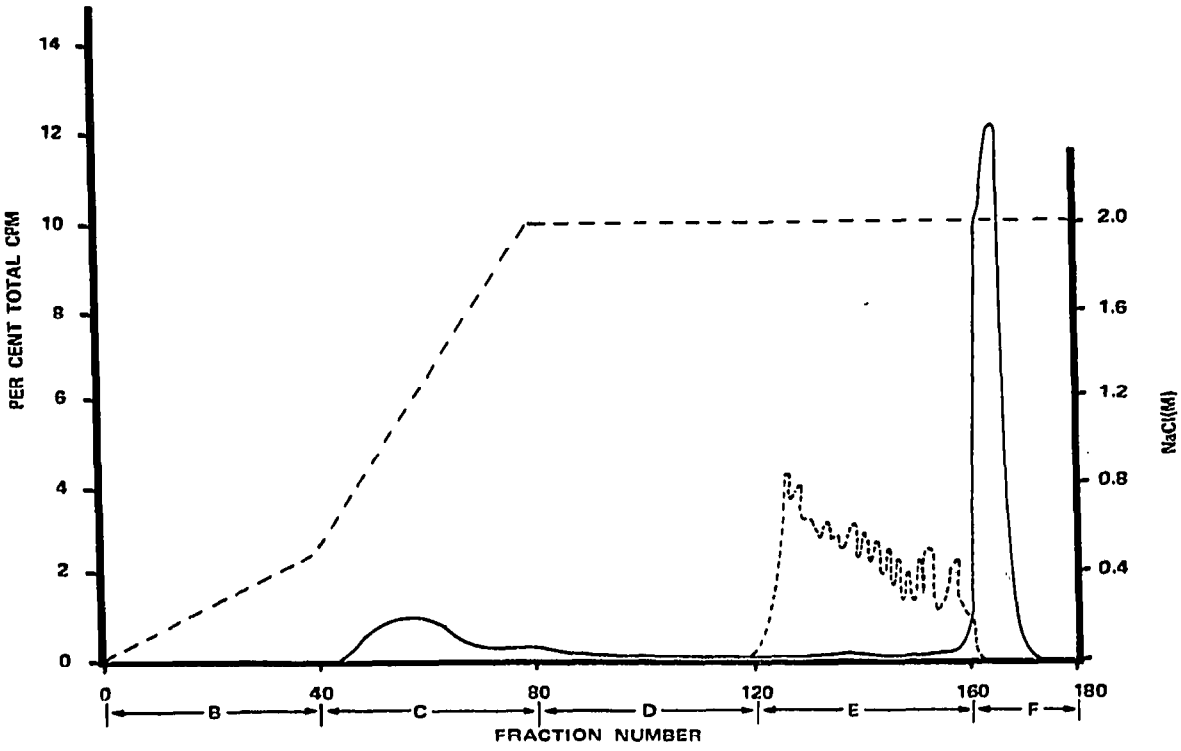

Fig. 5. Chromatography on DE-52 of non-dried ^{3}H-labelled "newly synthesized" P388F DNA (240 μg). Specific activity 9.56 μCi/mg. ———, Native DNA; – – –, heat-denatured DNA. The salt gradient is indicated as in Fig. 1. Total cpm, 8.15×10^5.

molarity (regions E and F) is all or part single-stranded on elution, and that a component of the original DNA which strongly adheres to the column is eluted in region F. The heterogeneity of the P388F DNA is best observed on denaturation, as indicated by the shape of the elution profile.

The effect of formaldehyde on native DNA and on DNA during heat denaturation was studied. Approximately 34 μg of high-specific-activity DNA in the presence of 1% formaldehyde was applied to the column in each case. Native DNA, treated with formaldehyde for 40 min prior to application to the column, produced only a slight sharpening of the peak at 0.8 *M* NaCl (Fig. 3). The relative amounts of peaks in regions C and F were approximately the same in this case, *viz.* 36.8 and 36.2%, respectively. The presence of 1% formaldehyde during heat denaturation, however, produced a marked change in the eluting position of the DNA (Fig. 4) and in the shape of the profile observed when compared to untreated, heat-denatured DNA (*cf.* Fig. 2). With formaldehyde present, most of the DNA is eluted in region B. It appears, therefore, that formaldehyde reduces the total charge distribution of denatured DNA, probably by its interaction with primary amino groups, so that the DNA adheres much less strongly to the column. With native DNA, amino groups of adenine, guanine and cytosine are not readily available for reaction with formaldehyde, which may be related to this effect. The fact that the fraction of native DNA

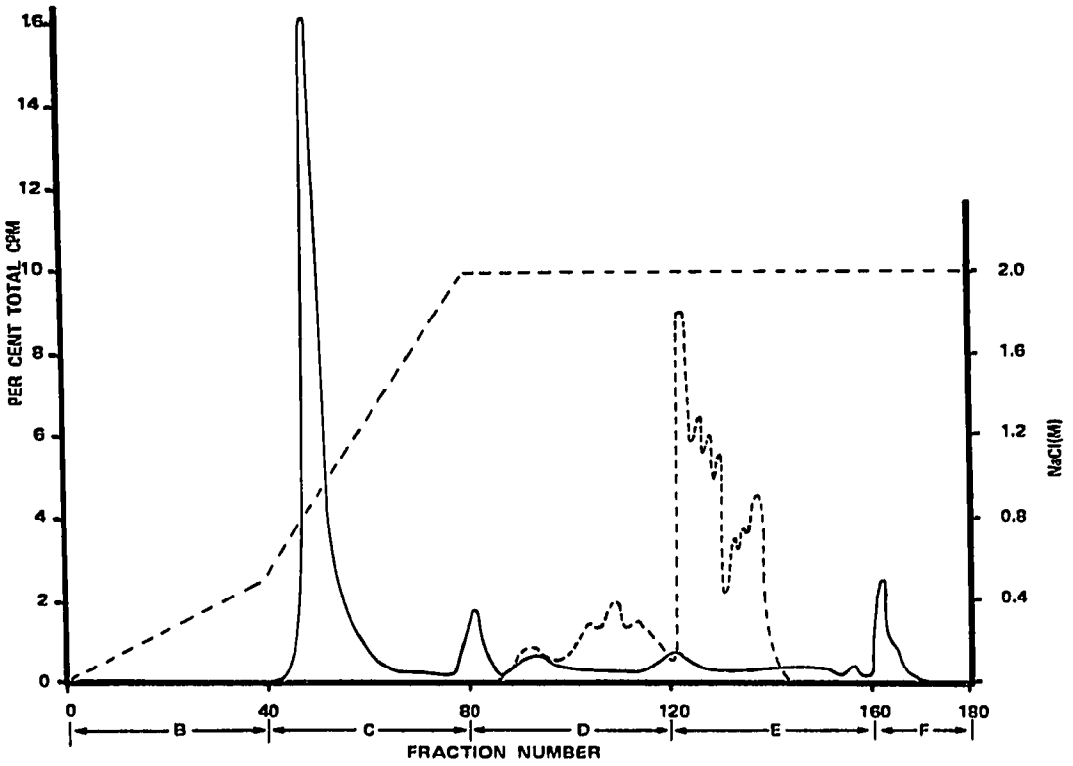

Fig. 6. Chromatography on DE-52 of non-dried ^{14}C-labelled "template" P388F DNA (442 μg). Specific activity 2.69 μCi/mg. ———, Native DNA; — — —, heat-denatured DNA. Total cpm, 1.69×10^6.

appears in region F and is unaffected by formaldehyde therefore makes it unlikely that it is a single-stranded DNA fraction, which is normally present in native DNA. It must represent a naturally occurring component with increased affinity for DEAE-cellulose either per se or brought about during the elution process. Attempts to elucidate the nature of the peak in region F are described below.

DEAE-cellulose chromatography of "newly synthesised" ^{3}H-labelled DNA

The shape of the profile obtained with newly synthesised DNA of relatively high specific activity (Fig. 5) is qualitatively similar to that of "template" labelled DNA (Fig. 2) but the amount of label within the gradients is different. In the case of newly synthesised DNA, 17.5% of labelled material is eluted with 0.8 *M* NaCl and 65% with 0.5 *N* NaOH, compared with 40.6 and 34.9%, respectively, with the "template" labelled DNA. The increase in the level of components in region F in newly synthesised DNA could be due to increased fragility or the presences of "nicks" in the DNA, leading to increased affinity to DEAE-cellulose. However, on denaturation (Fig. 5) the fraction in region F is lost completely and 92% of labelled material is found in region E.

DEAE-cellulose chromatography of "template" ^{14}C-labelled DNA

Because of the possibility of breakage and instability of P388F DNA due to the high level of tritium labelling[25,26], [^{14}C]thymidine was used. A marked increase in the level of double-stranded DNA eluting with 0.8 *M* NaCl in region C (Fig. 6) was observed (68.9%) and only 5.6% is eluted with 0.5 *N* NaOH (region F). The labelled materials from the peaks in regions C and F were taken for further analysis by alkaline sedimentation centrifugation (see below). Heat denaturation caused most of the DNA to be eluted in regions D and E (Fig. 6), with about 29% in region D and 67% in region E but none in region F, in contrast to the profile obtained with "template" ^{3}H-labelled DNA.

Effect of partial hydrolysis with pancreatic DNAase I

By treating a solution of "template" ^{14}C-labelled DNA (15 μg/ml) with pancreatic DNAase I, as described in Experimental, it can be seen (Fig. 7) that the effect of reducing the DNA to oligonucleotides is, as expected, to shift the radioactivity from regions C to B, to lower salt concentrations. Not all the original DNA was digested, confirmed by the presence of 42% of label still present in region C. The increase of 52% of labelled material in region B thus represents the extent of oligonucleotide production by this enzyme under these conditions.

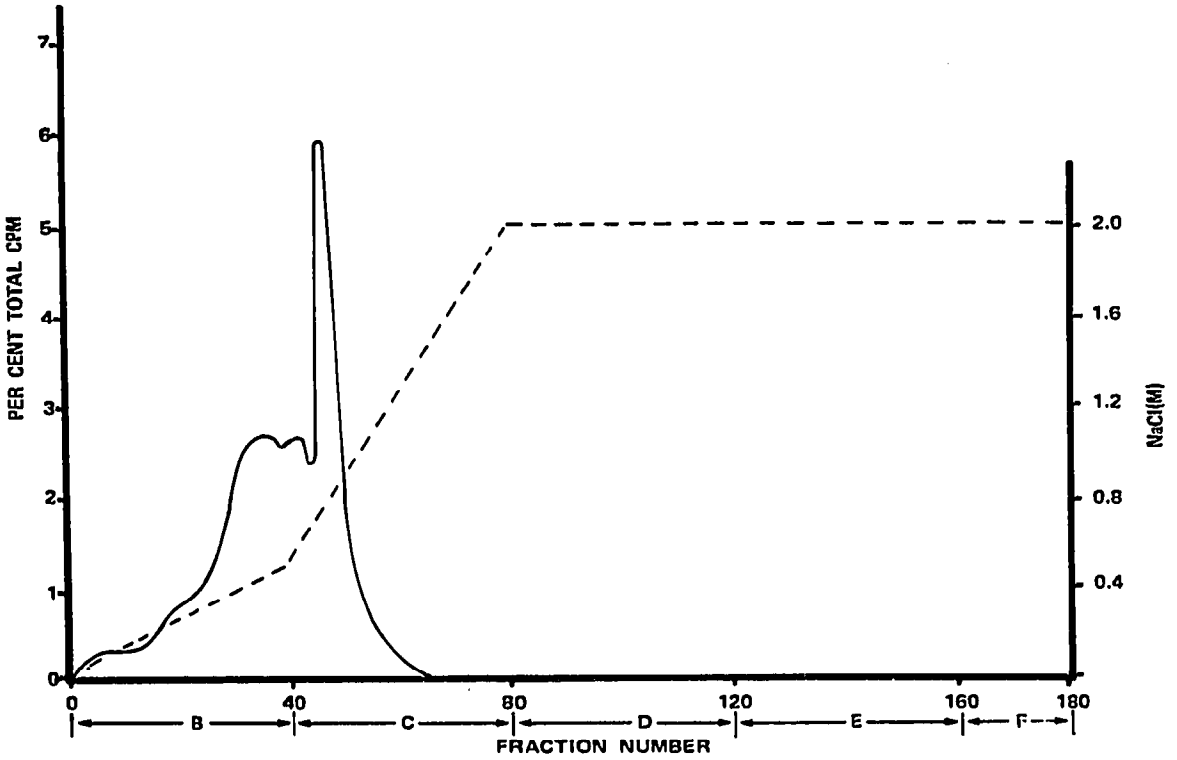

Fig. 7. Chromatography on DE-52 of non-dried ^{14}C-labelled "template" P388F DNA (45 μg) treated with pancreatic deoxyribonuclease I as described in Experimental. Specific activity, 2.69 μCi/mg. The salt gradient is indicated as in Fig. 1. Total cpm, 2.26×10^5.

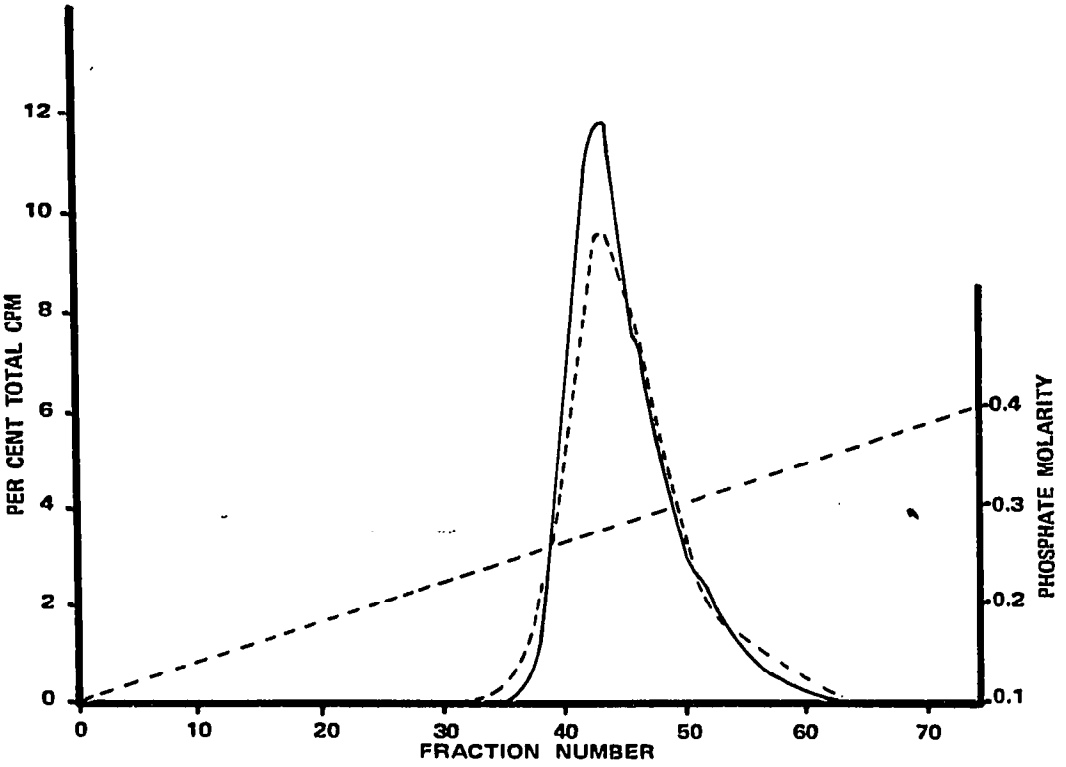

Fig. 8. Chromatography on hydroxyapatite of ^{3}H-labelled "template" P388F DNA. Specific activity, 67.4 μCi/mg. ———, Fraction C (3.0 μg DNA, total cpm, 4.64 × 10^{4}); - - - - -, native DNA. The molar concentration of potassium phosphate in the eluting buffer is indicated by a dashed line.

DEAE-cellulose chromatography of T4 phage DNA

The profile obtained with T4 phage ^{14}C-labelled DNA is practically identical to that of "template" ^{14}C-labelled P388F DNA, with approximately 70% of radioactive material eluting with 0.8 *M* NaCl. Thus, the five fold difference in the molecular weight of the DNA does not appear to affect the position of elution.

Hydroxyapatite chromatography

Hydroxyapatite column fractionation of native "template" ^{3}H-labelled P388F DNA indicates double-stranded DNA eluting in a single peak at 0.28 *M* phosphate (Fig. 8). The peak of radioactivity obtained from region C (0.8 *M* NaCl) of the DE-52 column separation (Fig. 3) was found to be coincident with the original native DNA when run on this column (Fig. 8), confirming its double-stranded nature. The peak from region F from the same DE-52 column yields a more complex elution profile (Fig. 9) on hydroxyapatite. The main bulk of the DNA is eluted at lower phosphate molarity than is double-stranded DNA, but not at a molarity as low as is single-stranded DNA. More DNA is also dispersed throughout the gradients. A smaller fraction is, however, evident at 0.10 *M* phosphate. Thus the material from region F would appear to be "native-like" even after alkaline elution from the DE-52 column.

The pattern obtained, however, could represent different degrees of renaturation on reintroduction into the neutral environment of the hydroxyapatite column. The original P388F DNA, following heat denaturation, yielded a complex profile on hydroxyapatite with two main peaks occurring (Fig. 9). These results are essentially in agreement with those obtained by Bernardi[3,4]. The main bulk of DNA is eluted around 0.18 *M* phosphate, corresponding to single-stranded DNA, but a large fraction is also observed in the "native"-like region, around 0.24–0.25 *M* phosphate. When the "native" DNA fraction (region C) is heat-denatured, it is eluted from a hydroxyapatite column around 0.18 *M* phosphate (Fig. 10). Thus, it would appear that the original DNA (Fig. 9) is indeed heterogenous in composition and that it is, at least partially, resolved in the DE-52 column. The fraction eluting at 0.18 *M* phosphate apparently originates from the peak in region C of the DE-52 column. The "native-like" component eluting at 0.24–0.25 *M* phosphate in the hydroxyapatite column must therefore originate from a component of the peak in region F from the DE-52 column. This latter component either possesses a markedly high resistance to denaturation (either heat or alkali) or is capable of a considerable degree of renaturation.

Alkaline sucrose gradient centrifugation

The alkaline sucrose gradient sedimentation pattern of "template" DNA labelled with [^{14}C]thymidine and corresponding fractions eluting from a DE-52

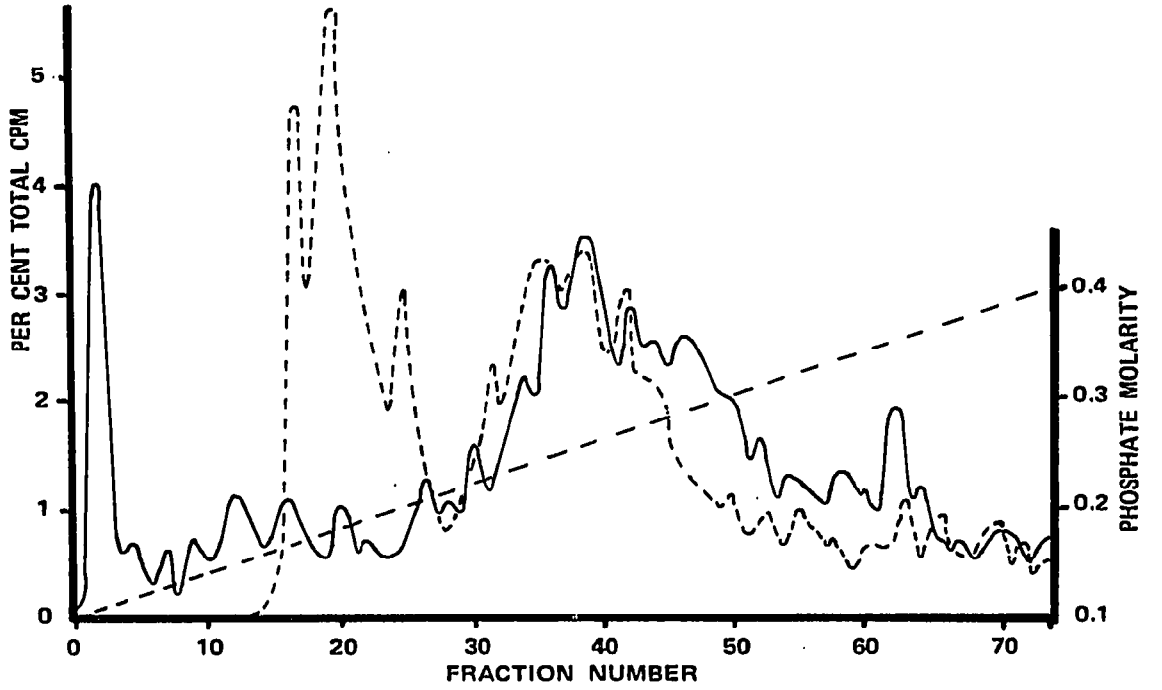

Fig. 9. Chromatography on hydroxyapatite of Fraction F (———) (total cpm, 1.13 × 10^4) of formaldehyde-treated DNA (Fig. 3) and of heat-denatured ^{3}H-labelled "template" DNA with a specific activity of 67.4 μCi/mg (— — —) (total cpm, 5.30 × 10^5). The phosphate molarity is indicated as in Fig. 8.

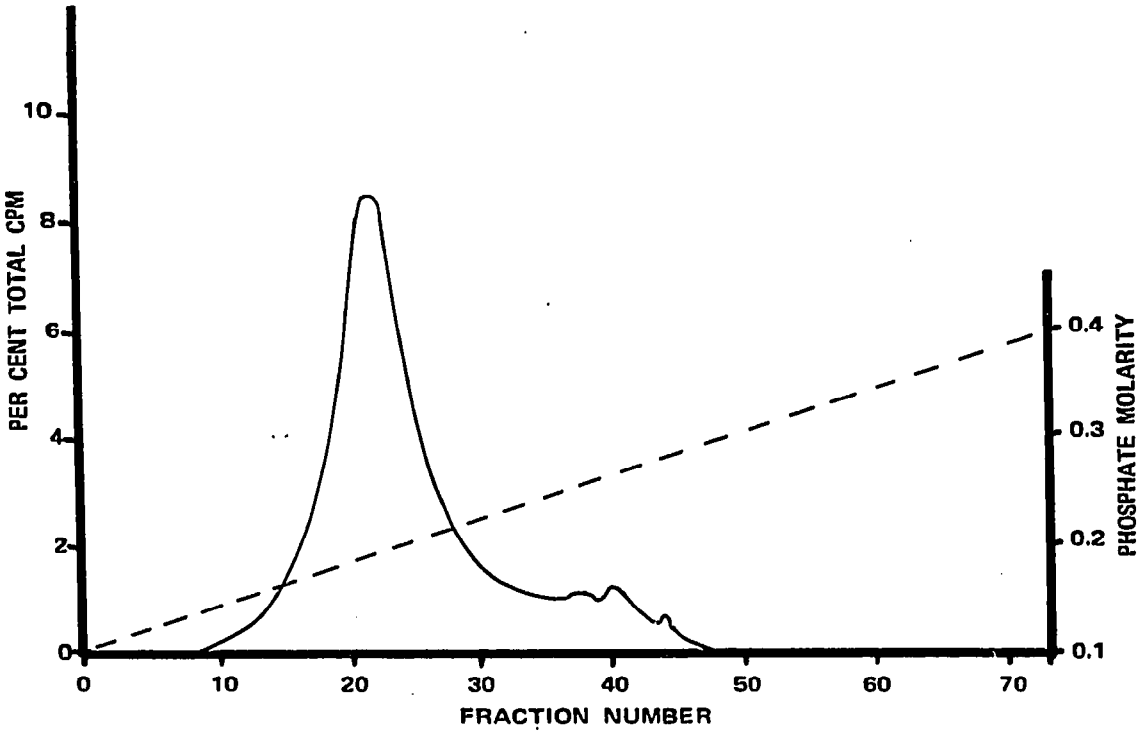

Fig. 10. Chromatography on hydroxyapatite of fraction C (0.92 μg) from experiments depicted in Fig. 3 following heat denaturation. The phosphate molarity is as indicated in Fig. 8. Total cpm, 1.39×10^4.

column (see Fig. 6) is shown in Fig .11. The peak from region C has approximately the same sedimentation coefficient as has the original DNA, whereas the fraction from region F has a slightly lower $S_{20,w}$ value. The peaks from region D contain a heterogeneous mixture of thymidine-labelled materials, whereas the peak in region E is more homogeneous. Calibration with phage T4 DNA indicates that peaks from regions D and E consist of small subunits in the 14–24S range of $S_{20,w}$ values.

DISCUSSION

The present results indicate that native DNA may be resolved into at least two main components by chromatography on DE-52, one at approximately 0.8 *M* NaCl and one after elution with 0.5 *N* NaOH. The peak at 0.8 *M* NaCl appears to represent the main bulk of double-stranded DNA. The DNA eluted by 0.5 *N* NaOH in 2 *M* NaCl is "native-like", as shown by hydroxyapatite fractionation, and has possibly a partially helical structure containing disordered regions in the original samples, but due to the alkaline elution it, appears as single-stranded DNA on DE-52 (peak F). This DNA, however, readily renatures to a "native-like" (possibly random-coil) configuration when subjected to chromatography on hydroxyapatite. The fact that a similar fraction is evident when denatured DNA is chromatographed directly

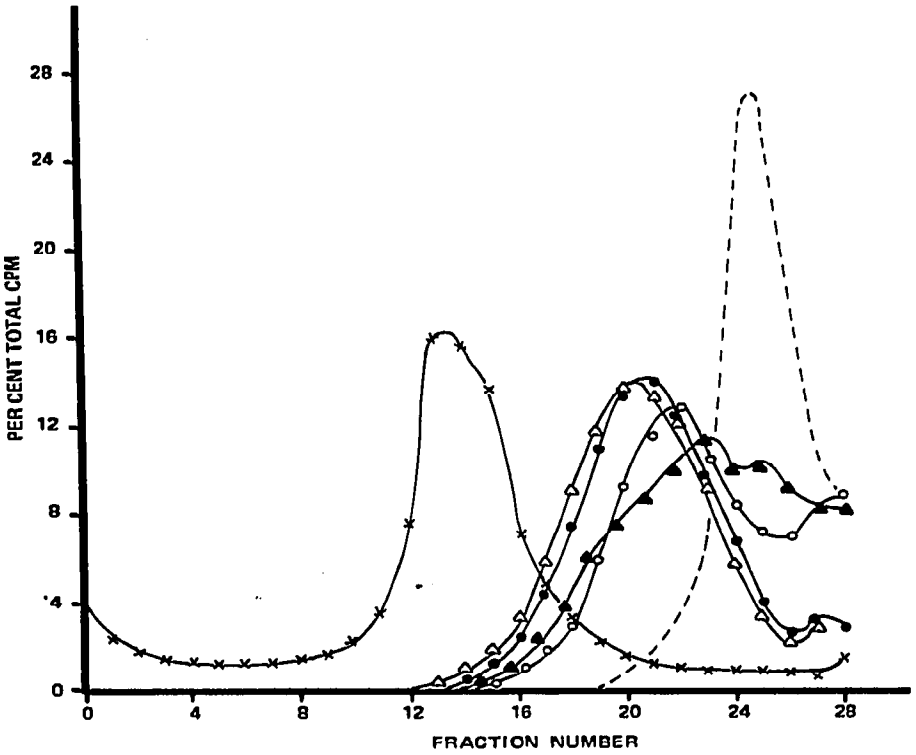

Fig. 11. Alkaline sucrose gradient sedimentation centrifugation of ^{14}C-labelled "template" P388F DNA and the corresponding fractions isolated from a DE-52 column (see Fig. 6). ×—×, ^{14}C-Labelled DNA from T4 phage (mol. wt., 6.0×10^7; total cpm, 3.47×10^4; △—△, ^{14}C-labelled "template" P388F DNA (total cpm, 5.26×10^4); ●—●, fraction C (total cpm, 2.89×10^4); ▲—▲, fraction D (total cpm, 1.02×10^4); ----, fraction E (total cpm, 3.38×10^4); ○—○, faction F (total cpm, 1.47×10^4).

on hydroxyapatite suggests that this component of DNA might have been formed by reassociation of some of the complementary strands of DNA after denaturation.

The presence of formaldehyde during the denaturation of DNA can be shown to have a profound effect on the elution profile compared with that of untreated denatured DNA. Elution of formaldehyde-treated DNA at such low salt concentration may be caused by the reduction of charge by hydroxymethylation of available bases of denatured DNA available for absorption on to DEAE-cellulose. The increased homogeneity of the fractionation in the presence of formaldehyde would also support the view that the varying distribution of charges available on the original DNA may be leading to the heterogeneity seen.

The amount of labelled material eluted by 0.5 *N* NaOH in region F increases relative to that eluted by 0.8 *M* NaCl in region C when the isolated DNA has been taken to dryness, and when "newly synthesised" as opposed to "template" DNA is fractionated. Both these DNA preparations could be expected to be more fragile, the former having lost important structural water molecules and the latter may contain "nicks" in the DNA prior to ligase activity involved in polynucleotide synthesis[27,28]. The effect of labelling of the DNA with [^{3}H]- as opposed to [^{14}C]thymidine also

seems to enhance this increased affinity for DEAE-cellulose. A correspondingly more alkaline pH is also required for elution of denatured ^{3}H-labelled DNA as opposed to ^{14}C-labelled DNA.

The use of an advanced DEAE-cellulose column does offer a potentially valuable means of a detailed analysis of DNA structure from the point of view of both secondary structure and topographical features. The excellent reproducibility of the procedure in repeated runs suggests that even finer resolution of DNA species will be possible.

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