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An Analysis of the Bovine Genome by Cs_2SO_4–Ag^+ Density Gradient Centrifugation

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Calf DNA preparations having molecular weights of 5 to 7×10^6 have been fractionated by preparative Cs_2SO_4–Ag^+ density gradient centrifugation into a number of components. These may be divided into three groups: (1) the main DNA component (1·697 g/cm³; all densities quoted are those determined in CsCl density gradients), the 1·704 and 1·709 g/cm³ components form about 50, 25 and 10% of the genome, respectively; they are characterized by having symmetrical CsCl bands and melting curves, both of which have standard deviations close to those of bacterial DNAs of comparable molecular weight, and by their G + C contents being equal to 39, 48 and 54%, respectively; after heat-denaturation and reannealing, their buoyant densities in CsCl are greater than native DNA by 12, 10 and 3 mg/cm³, respectively.

(2) The 1·705, 1·710, 1·714 and 1·723 g/cm³ components represent 4, 1·5, 7 and 1·5% of the DNA, respectively, and exhibit the properties of "satellite" DNAs; their CsCl bands and melting curves have standard deviations lower than those of bacterial DNAs; after heat-denaturation and reannealing, their buoyant densities are identical to native DNA, except for the 1·705 g/cm³ component, which remains heavier by 5 mg/cm³; in alkaline CsCl, only the 1·714 g/cm³ component shows a strand separation.

(3) A number of minor components, forming 1% of the DNA, have been recognized, but they have not been investigated in detail; two of them (1·719 and 1·699 g/cm³) might correspond to ribosomal cistrons and mitochondrial DNA, respectively.

1. Introduction

When centrifuged to equilibrium in CsCl density gradients, DNAs from higher organisms show broad, asymmetrical bands (Meselson *et al.*, 1957) often accompanied by minor, satellite bands (Kit, 1961; Sueoka, 1961), in contrast with viral and prokaryotic DNAs, which show unimodal, symmetrical bands. A method with a much higher resolving power is the centrifugation to equilibrium in Cs_2SO_4–Ag^+ density gradients: owing to a differential binding of silver ions, components of eukaryotic DNAs, which are difficult or impossible to separate in the classical CsCl density gradients, can be resolved by this technique (Corneo *et al.*, 1968).

We report here a detailed analysis of the bovine genome at a size level of 5 to 7×10^6 molecular weight, using as the main tool preparative centrifugation in Cs_2SO_4–Ag^+

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density gradients. This approach has enabled us to resolve the bovine genome into a number of DNA components†, seven of which have been characterized by their physical and chemical properties.

2. Materials and Methods

(a) *Calf thymus DNA*

This was preparation CTR2, described elsewhere (Soave *et al.*, 1973). Its sedimentation coefficient was equal to 22·6 S, corresponding to a molecular weight of 5×10^6 (Prunell & Bernardi, 1973).

(b) *Calf liver DNA*

This was obtained according to Kay *et al.* (1952) and was further purified by chromatography on hydroxyapatite, in order to remove some material (presumably from degraded RNA) eluting before DNA; in a typical experiment, 60 ml of the DNA solution ($A_{260} = 2·2$) in 3 M-KCl, 0·2 M-potassium phosphate buffer, pH 6·8, were loaded on a 2 cm × 40 cm column equilibrated with the same solvent; elution was carried out with a molarity gradient of 0·2 to 0·45 M-potassium phosphate buffer, pH 6·8, 3 M in KCl throughout. The sedimentation coefficient of calf liver DNA was equal to 25 S (mol. wt $= 7 \times 10^6$).

(c) *Density gradient preparative centrifugation*

DNA samples to be used in CsCl preparative centrifugations were dialysed against 0·005 M-NaCl, 0·01 M-Tris buffer, pH 7·5. Solid CsCl (Suprapur, Merck, Darmstadt, Germany) was added to the solutions to give densities of 1·715 or 1·725 g/cm^3. DNA samples to be used in Cs_2SO_4 preparative centrifugations were dialysed against 0·005 M-sodium tetraborate, pH 9·2, or against borate solutions prepared by titrating 0·01 M-boric acid with 1 N-NaOH to the desired pH value, and by diluting it with one volume of water; 4×10^{-3} M-$AgNO_3$ was added to reach a Ag^+/DNA-P molar ratio, r, equal to 0·35, except where otherwise stated; finally, solid Cs_2SO_4 (Suprapur, Merck) was added to give a density of 1·5 g/cm^3. The molar ratio r was determined by using a ϵ(P) value of 6600, and knowing that all Ag^+ added to the solution was bound by DNA under the experimental conditions used (unpublished results). A Zeiss (Oberkochen, Germany) immersion refractometer was used to determine the refractive index, and therefore the density, of CsCl and Cs_2SO_4 solutions.

Centrifugations were carried out in a Spinco L265B preparative ultracentrifuge using type 30 or 65 aluminium rotors. Centrifugation times and speed, and DNA concentrations are given in the Figure legends. Nitrocellulose or polyallomer tubes were completely filled with the DNA solutions in the case of CsCl density gradients, whereas they were only partially filled (to 25 to 26 ml in type 30 rotor, and to 11 ml in type 65 rotor) in the case of Cs_2SO_4–Ag^+ density gradients; in this case a layer of paraffin oil (Merck) was used to protect the solution from contact with the tube cap. After centrifugation, tubes were emptied using a stainless steel needle reaching the bottom of the tube; a hollow cylinder screwed onto the tube cap was used to guide the needle. The centrifuged solution was pumped out using a Technicon (Chauncey, N.J.) peristaltic pump operated at a flow rate of 12 or 20 ml/h, according to whether small or large tubes were used; the solution was pumped through the 0·3-cm cell of an LKB Uvicord (Stockholm, Sweden) and its absorbance at 253·7 nm was recorded. Fractions were collected either manually or automatically using an LKB Ultrorac fraction collector operated on a time basis. Fractions obtained from Cs_2SO_4–Ag^+ density gradient centrifugations were dialysed against 2 or 3 M-NaCl before any further treatment, in order to remove Ag^+.

(d) *Analytical density gradient experiments*

These were done as previously described (Bernardi *et al.*, 1970,1972) using a titanium An-G rotor, 12-mm double-sector cells and a Spinco model E ultracentrifuge equipped

† For the terminology used in the present article see Discussion, section (c).

with a monochromator, an electric scanner and a multiplexer. Scanning was at 265 nm and at a slit width of 0·12 mm. A scanning speed of 1·25 cm/min was used with a chart speed of 30 cm/min; this corresponds to an enlargement of 24-fold along the abscissa. Scannings at a speed of 5·63 cm/min, corresponding to an enlargement on the abscissa of only about 5-fold, were used in experiments shown in all Figures, except for Figs 2, 7 and 8. DNA samples were prepared for neutral CsCl density gradients as described for the preparative centrifugation; in the case of alkaline CsCl density gradients, the DNA solutions were first adjusted to a pH of about 12·5 by addition of 1 N-NaOH; after centrifugation, solutions were neutralized by addition of 0·5 vol. of 2 M-sodium phosphate buffer, pH 6·8, and then dialysed against appropriate solvents for further work.

(e) *Sedimentation velocity experiments*

These were done by the band centrifugation method, with Vinograd 30-mm, single-sector cells, as described elsewhere (Prunell & Bernardi, 1973).

(f) *Base composition*

This was determined, after enzymic digestion to nucleosides, using BioGel P2 or Aminex A6 columns (Carrara & Bernardi, 1968; Piperno & Bernardi, 1971; Thiery *et al.*, 1973). The 5-methylcytidine content was estimated on the basis of the recording at 271 and 300 nm of the cytidine peak; methylcytidine is very slightly retarded compared to cytidine on the Aminex column.

(g) *Absorbance–temperature*

Experiments were done using equipment to be described elsewhere (A. Prunell & G. Bernardi, unpublished data); in a solvent of 0·1 × SSC (SSC is 0·15 M-NaCl, 0·015 M-sodium citrate, pH 7·2). The DNA concentration varied, in different experiments, from 10 to 25 μg/ml.

(h) *Renaturation experiments*

DNA denaturation was carried out by heating DNA solution (3 to 10 μg/ml in 0·1 × SSC) at 100°C for 10 min. DNA reannealing was carried out near the optimum conditions of renaturation (T_m − 25 deg. C) in 2 × SSC for 5 h.

3. Results

(a) *Fractionation in calf DNA in Cs_2SO_4–Ag^+ density gradients*

Three experiments carried out at pH 9·2 will be described here.

(i) At $r = 0·3$, the densitogram of calf thymus DNA (Fig. 1) shows a main peak exhibiting three shoulders (arrows 1 to 3) on its light side, and two lighter, minor peaks (arrows 4 to 5). Fractions 2 to 4 were re-banded in Cs_2SO_4–Ag^+ density gradients under centrifugation conditions similar to those used in the first centrifugation, whereas fraction 1 was re-run in a preparative CsCl density gradient (Fig. 1(b)). Analytical CsCl density gradients were run on subfractions obtained in the second series of centrifugations (Fig. 1(b)), and showed either asymmetrical or multiple bands. Some subfractions therefore, were re-banded in CsCl preparative density gradients (Fig. 1(c) and (d)); analytical CsCl density gradients carried out on cuts from these preparative centrifugations showed that symmetrical or nearly symmetrical peaks were obtained for DNA components having buoyant densities equal to 1·714, 1·709, 1·723 and 1·705 g/cm³. These components were apparently responsible for the second and third shoulders on the light side of the main peak (arrows 2 and 3; Fig. 1(a)) and for the two light peaks (arrows 4 and 5), respectively; the first shoulder of the main peak (arrow 1, Fig. 1(a)) was due to the 1·704 g/cm³ component, as shown by the experiments described below.

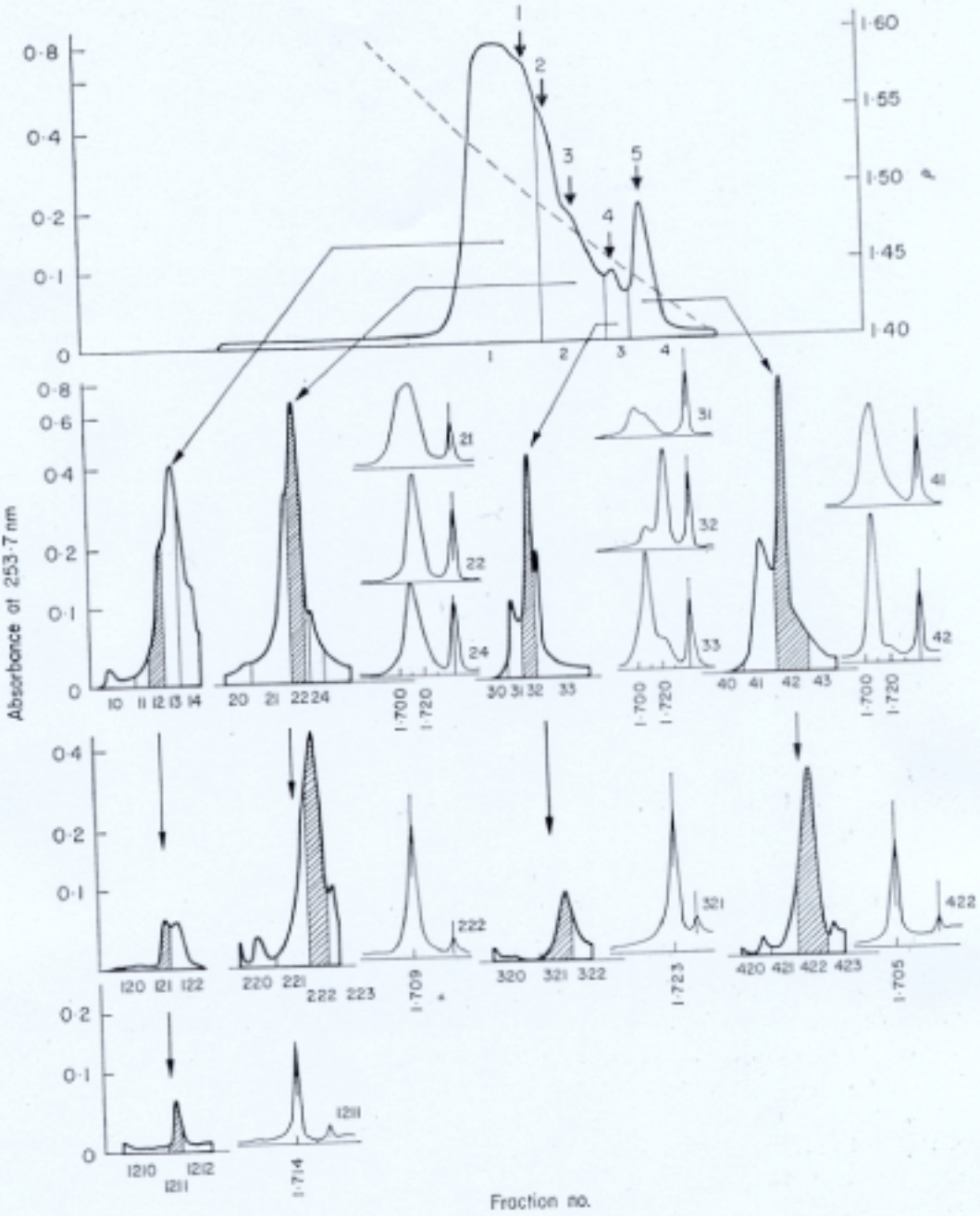

FIG. 1. Analysis of calf thymus DNA components using preparative Cs_2SO_4–Ag^+ density gradient centrifugation. A calf thymus DNA solution ($A_{260} = 1{\cdot}25$) in 0·0044 M-tetraborate buffer pH 9·2, containing $AgNO_3$ ($r = 0{\cdot}3$) and Cs_2SO_4 ($\rho = 1{\cdot}5$ g/cm^3) was centrifuged in a type 30 rotor at 29,000 revs/min for 80 h, and at 26,000 revs/min for an additional 20 h to reduce the slope of the gradient (Hatch & Mazrimas, 1970). The material obtained was separated into fractions 1 to 4. Corresponding fractions obtained from 12 tubes, simultaneously centrifuged, were pooled together. Fractions 2, 3 and 4 were adjusted to a density of 1·45 g/cm^3 with a Cs_2SO_4 solution (A_{260} values were 0·48, 0·25 and 0·60, respectively) and re-run in a type 30 rotor at 29,000

The preparative centrifugation of calf thymus DNA in a Cs_2SO_4–Ag^+ density gradient, in Figure 1, showed that a fine resolution of components was obtained on the light side of the main band, as indicated by the presence of a number of shoulders and peaks in the densitogram. It was clear, however, that much of the actual resolution of components was lost because the densitogram was separated into four fractions only.

(ii) In a second experiment (Fig. 2) 17 fractions were collected from a single tube and submitted to analytical CsCl density gradient centrifugation; in this case the r value was 0·35 (see section (b) below) and the DNA concentration was lower; under these conditions, the second lightest peak (arrow 4) of Figure 1 was resolved into two peaks.

At the extreme left (heavy side) of the densitogram, two fractions (6 and 7) showed broad bands in CsCl (1·697 and 1·698 g/cm^3) and a trace component having a density of 1·714 g/cm^3. The buoyant densities of fractions corresponding to the main band (8 and 10) increase from $1{\cdot}696_5$ to $1{\cdot}697_8$ and $1{\cdot}699_8$ g/cm^3; considering the width of the cuts and their position across the densitogram, this shift seems to be due to the increasing influence of the neighbouring 1·704 g/cm^3 component (see also the rebanding experiments in Fig. 3(b), below), although a possible existence of a component of density 1·700 g/cm^3 cannot be ruled out. Fractions 11 and 12 show a component with a density of 1·704 g/cm^3 which is accompanied by increasing amounts of 1·709 and 1·714 g/cm^3 components; the latter shows a sharp, characteristic peak in fraction 12. Fractions 14 to 22 are characterized by the presence of several heavy components having densities of 1·710 (fractions 14 to 16), 1·723 (fractions 17 to 18) and 1·705 g/cm^3 (fractions 21 to 22). A minor component (1·719 g/cm^3) is also present in fractions 21 and 22 and two other minor components (1·699 and 1·716 g/cm^3) are present in fractions 13 to 17 and 15 to 17, respectively. The experiment shown in Figure 2 confirmed that a fine resolution of components does indeed take place in the gradient, which allowed us to obtain a reasonable estimate of the amounts of different DNA components in calf thymus DNA.

The Table shown in Figure 2 gives the percentage and the density of the components present in each fraction. It is possible to associate well-defined buoyant density values in CsCl with specific DNA components, in spite of the slight differences in their measured densities caused by the presence of neighbouring components, if account is taken (1) of the typical density shifts undergone by the components as a consequence of pH changes in Cs_2SO_4–Ag^+ density gradients (see section (b) below) and (2) of analytical experiments carried out on a very large number of fractions derived from different preparative Cs_2SO_4–Ag^+ density gradients, comprising rebandings of particular fractions. The data of the Table have been presented so as to make the identification easier of given DNA components; for instance, the 1·709, 1·710 and 1·709 g/cm^3 components present in fractions 15 to 17 all belong to the

revs/min for 90 h and at 22,000 revs/min for an additional 18 h. Fractions 1 and subfractions 12, 22, 32 and 42 were dialysed first against 3 M-NaCl and then against 0·005 M-NaCl, 0·01 M-Tris buffer, pH 7·5, and adjusted to a density of 1·725 g/cm^3 with solid CsCl, (A_{260} values were 0·75, 0·06, 0·53, 0·10 and 0·25, respectively); solutions were centrifuged in a type 30 rotor at 25,000 revs/min for 108 h. Subfraction 121 was adjusted to a density of 1·725 g/cm^3 with CsCl (A_{260} was 0·03) and centrifuged in a type 65 rotor at 40,000 revs/min for 40 h.

Some of the analytical CsCl centrifugations carried out on the fractions are also shown (fine-line drawings).

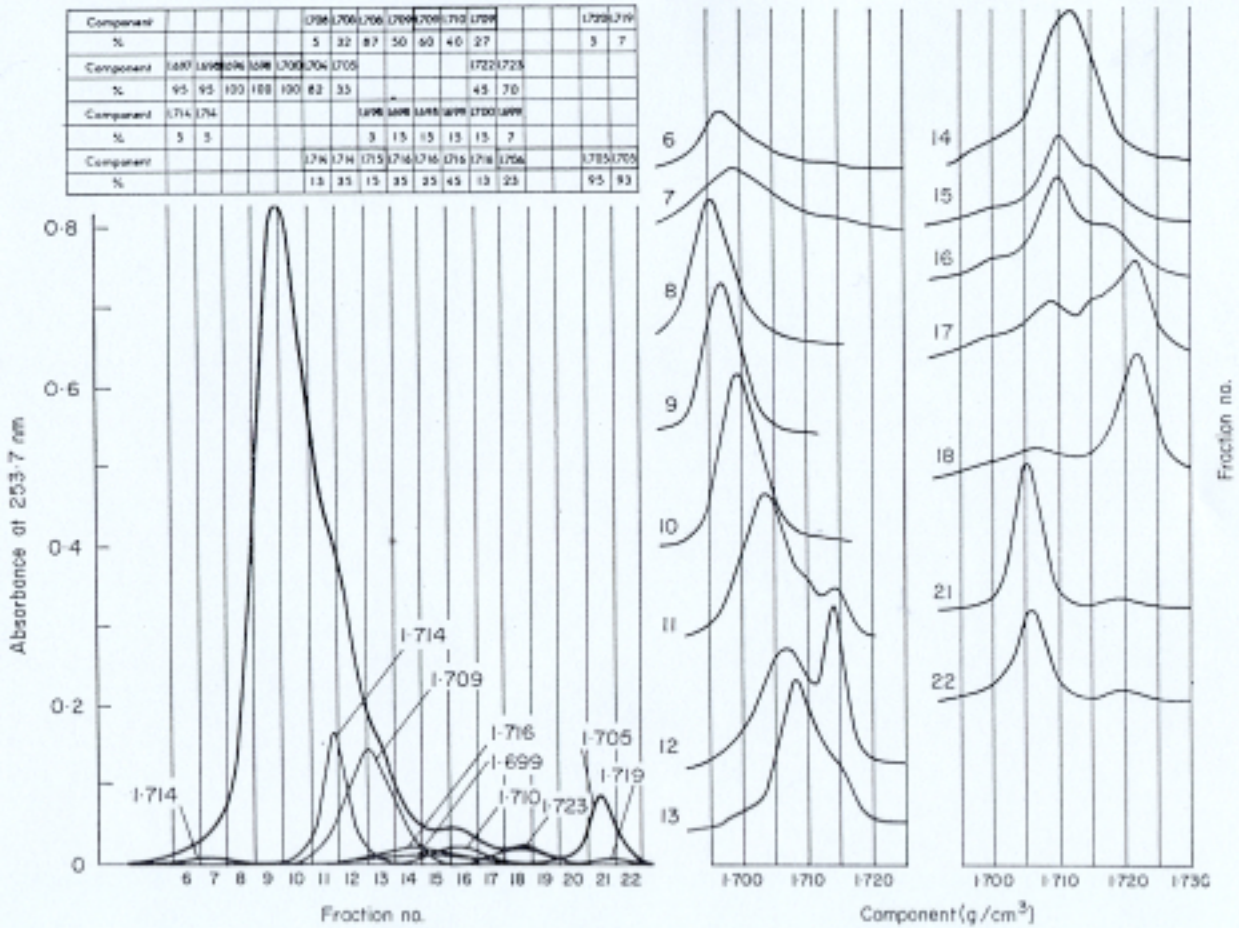

FIG. 2. Analysis of calf thymus DNA components using preparative Cs_2SO_4–Ag^+ density gradient centrifugation. A calf thymus DNA solution (A_{260} = 0·54) in 0·0044 M-tetraborate buffer pH 9·2, containing $AgNO_3$ (r = 0·35) and Cs_2SO_4 (ρ = 1·495 g/cm³) was centrifuged in a type 30 rotor at 25,000 revs/min for 90 h.

The analytical CsCl density gradient centrifugations (expanded-scale scannings) shown on the right concern 1-ml fractions obtained from the preparative run. The relative areas of the peaks corresponding to different DNA components are reported in the Table (insert; heavier line framing was used in order to help identify the buoyant densities belonging to the same components). On the basis of these values, the peaks corresponding to the DNA components were traced under the densitogram on the left, after normalizing their percentages to the total amount of DNA present in each fraction.

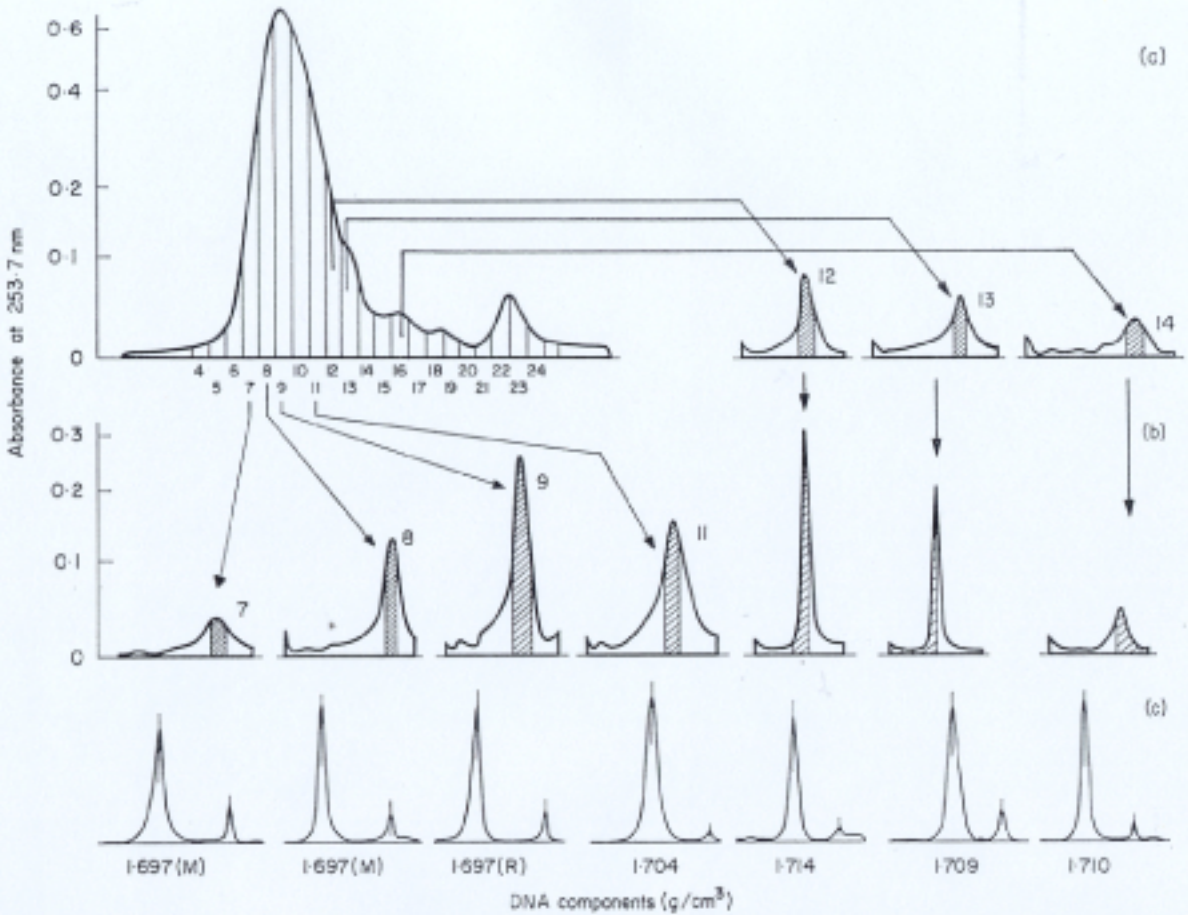

FIG. 3. Analysis of calf thymus DNA components using preparative Cs_2SO_4–Ag^+ density gradient centrifugation.

A calf thymus DNA solution ($A_{260} = 0{\cdot}55$) in 0·0044 M-tetraborate buffer, pH 9·2, containing $AgNO_3$ ($r = 0{\cdot}35$) and Cs_2SO_4 ($\rho = 1{\cdot}5$ g/cm³) was centrifuged in a type 30 rotor at 25,000 revs/min for 100 h. Fractions of 0·8 ml were collected from each tube; corresponding fractions were pooled (see text for the pooling procedure).

Fractions 7, 8, 9, 11, 12, 13 and 16 (A_{260} values were 0·095, 0·150, 0·290, 0·210, 0·170, 0·055 and 0·045, respectively) were dialysed first against 3 M-NaCl and then against 0·005 M-NaCl, 0·01 M-Tris buffer, pH 7·5, and adjusted to a density of 1·715 g/cm³ with solid CsCl; they were then centrifuged in a type 30 rotor at 25,000 revs/min for 75 h and at 22,000 revs/min for an additional 15 h.

Subfractions derived from fractions 12, 13 and 16 (A_{260} values were 0·140, 0·080 and 0·024, respectively) were centrifuged once more in a CsCl density gradient ($\rho = 1{\cdot}715$ g/cm³) in a type 65 rotor, at 40,000 revs/min for 40 h.

Analytical CsCl centrifugations are shown (fine-line drawings) for the central fractions (hatched areas) of the densitograms of Fig. 3(b).

1·710 g/cm³ component, whereas the 1·708, 1·708, 1·708 and 1·709 g/cm³ components of fractions 11 to 14 all belong to the 1·709 g/cm³ component.

(iii) The encouraging results shown in Figure 2 indicated that this procedure could be used on a preparative scale in order to obtain DNA components other than those prepared in the experiments shown in Figure 1. Figure 3 shows a typical densitogram, in which the material was collected into 22 instead of 17 fractions. Fractions deriving from ten tubes centrifuged simultaneously were pooled together on the basis of their position in the densitogram; pooling was restricted to the 4 to 5 fractions with similar values at A_{260}. The pooled fractions were submitted to a preparative CsCl density gradient centrifugation, a procedure which practically reverses the order of DNA components in the densitogram (see section (b) below). It should be noticed that the preparative CsCl patterns (Fig. 3(b)) have a greater resolving power than the analytical ones (Fig. 2), and also that the non-linear absorbance ordinate of Figure 3(b) exaggerates the apparent amount of material present on the sides of the peaks. As shown in Figure 3(c), the central fractions of the CsCl preparative densitograms showed unimodal, symmetrical peaks in the analytical centrifugations. Fractions 7 to 9 were mainly, or only, formed by a component having a density of 1·697 g/cm³; fraction 11 was mainly formed by a 1·704 g/cm³ component, though a contamination by the 1·714 g/cm³ component was present on its heavy side; fraction 10 (not shown) was mainly formed by material showing a density of 1·700 g/cm³, like fraction 10 of Figure 2; this material was also present in the light side of fraction 9. Not shown in Figure 3(c) are the results concerning fractions 18 to 19 and 22 to 24, corresponding to the 1·723 and 1·705 g/cm³ components, respectively (see Fig. 2), since these fractions were pooled with equivalent fractions from other preparations. It is evident that the procedure used in the experiment shown in Figure 3 represents a very marked improvement over that of the one shown in Figure 1, particularly in the following respects: (a) a new component, banding at 1·710 g/cm³, was isolated; (b) the 1·714 g/cm³ component was obtained in higher yield and with a smaller number of centrifugations; (c) the 1·709 g/cm³ component was obtained free from contaminating 1·710 g/cm³ component; (d) the 1·697 and 1·704 g/cm³ components were obtained free from each other.

(b) *Attempts to resolve other DNA components*

(i) Early eluting calf thymus DNA fractions from a hydroxyapatite chromatogram (Fig. 4(a)), when analysed by the Cs_2SO_4–Ag^+ technique (Fig. 4(b)), showed an enrichment (Fig. 4(c)) in the 1·704, 1·705, 1·710, 1·714 and 1·723 g/cm³ components. New components were not seen in any of the chromatographic fractions shown in Figure 4, nor in those obtained when elution was carried out in the presence of 3 M-KCl, a condition under which mouse satellite DNA shows a drastic shift in its chromatographic behaviour (unpublished results from our laboratory).

(ii) No new component was found when the Cs_2SO_4–Ag^+ method was used at different pH and r values. (a) The banding positions of DNA components changed greatly according to the pH used (Fig. 5): (i) a pH increase from 9·2 to 10·3 caused the 1·709 and 1·714 g/cm³ components to exchange their positions; (ii) at pH 7·9, the 1·714, 1·723, 1·709, 1·710 and 1·704 g/cm³ components appeared on the heavy side of the main peak, in that order, whereas the 1·705 and 1·719 g/cm³ components remained on

the light side. The complexity of the pH effect is illustrated in Figure 6(a). (b) When considering the effect of the r value (Fig. 6(b)), it was observed that: (i) the slope of the ρ *versus* r plot of the main DNA component is about 35 mg/cm^3 per 0·1 increase in the r value; this result is close to those reported by Jensen & Davidson (1966) at pH 8·0; (ii) the 1·705 and 1·719 g/cm^3 components are very peculiar in that they do not bind any silver up to r values equal to 0·3; (iii) when increasing the r value from

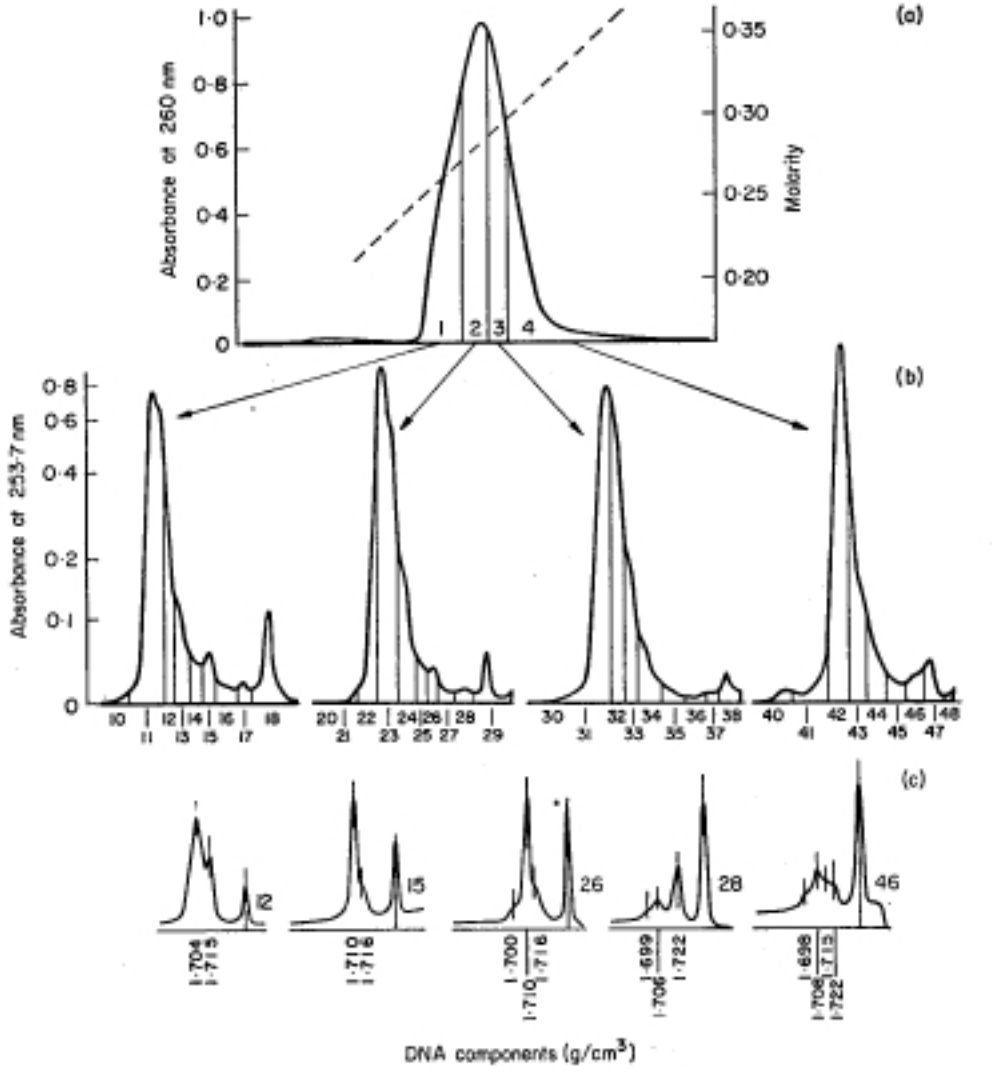

FIG. 4. Analysis of chromatographic fractions of calf thymus DNA by preparative Cs_2SO_4–Ag^+ density gradient centrifugation.

50 ml of a DNA solution ($A_{260} = 2·05$) in 0·2 M-sodium phosphate, pH 6·8, were loaded on a 2 cm × 45 cm hydroxyapatite column. Elution was done with a molarity gradient (0·2 to 0·4 M) of 350+350 ml of sodium phosphate pH 6·8; flow-rate was 40 ml/h (a).

The 4 fractions into which the chromatogram was split were centrifuged in 0·0044 M-tetraborate containing $AgNO_3$ ($r = 0·35$) and Cs_2SO_4 ($\rho = 1·5$) in a type 30 rotor at 25,000 revs/min for 85 h. A_{260} values of DNA solutions was 0·4 (b).

Some subfractions from the preparative centrifugation were banded in analytical CsCl density gradients (c).

0·35 to 0·40, the 1·710 and 1·723 g/cm³ components exchange their position, and the 1·719 g/cm³ component becomes lighter than the 1·705 g/cm³ component; (iv) the extrapolations of all curves (not shown) to r equals zero are very close to each other, as expected from the slight dependence of DNA buoyant density upon G + C contents in these gradients (Erikson & Szybalski, 1964).

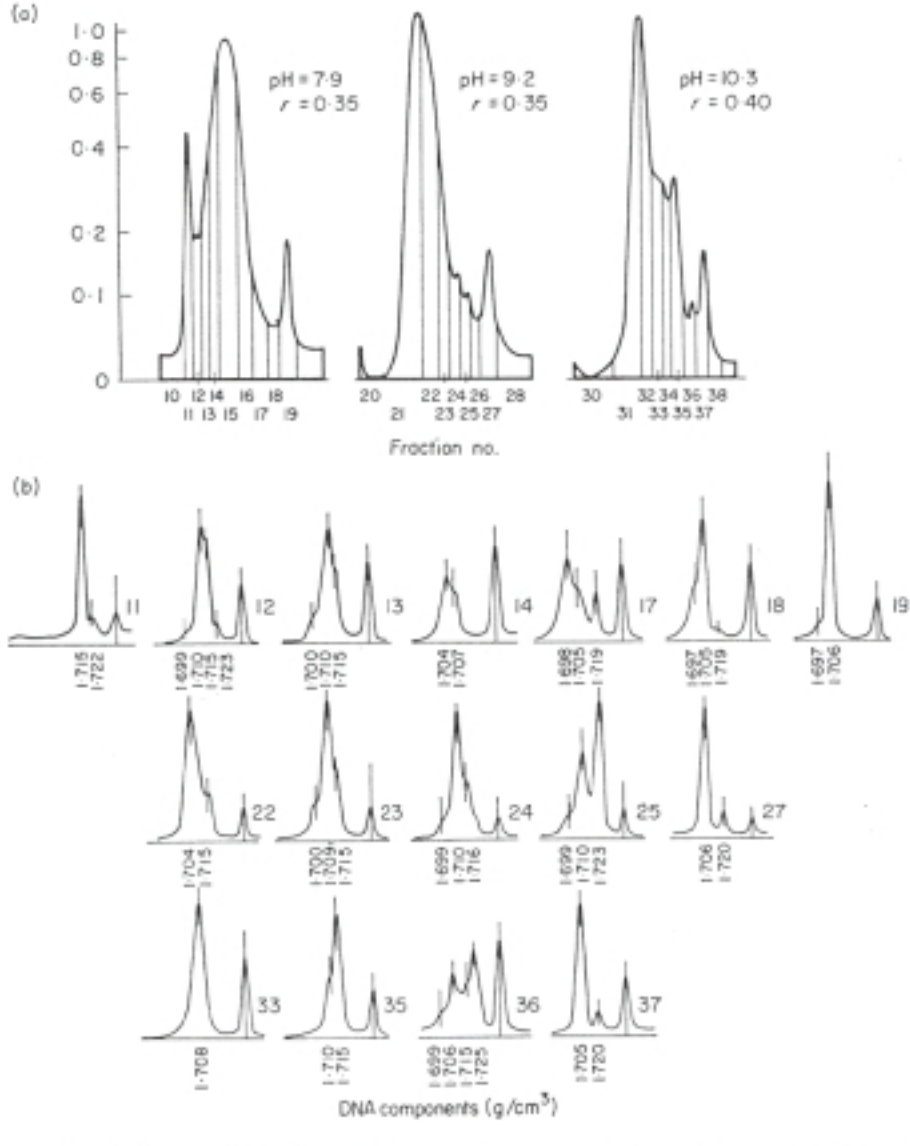

FIG. 5. (a) Analysis of calf liver DNA components using preparative Cs_2SO_4–Ag^+ density gradient centrifugation at 3 different pH values.

Calf liver DNA solutions (A_{260} = 0·83) in 0·0044 M-boric acid adjusted to the indicated pH and containing $AgNO_3$ (r values are indicated in the Figure) and Cs_2SO_4 (ρ = 1·5 g/cm³) were centrifuged in a type 65 rotor at 35,000 revs/min for 42 h.

(b) Analytical CsCl centrifugations carried out for some fractions are also shown (fine-line drawings).

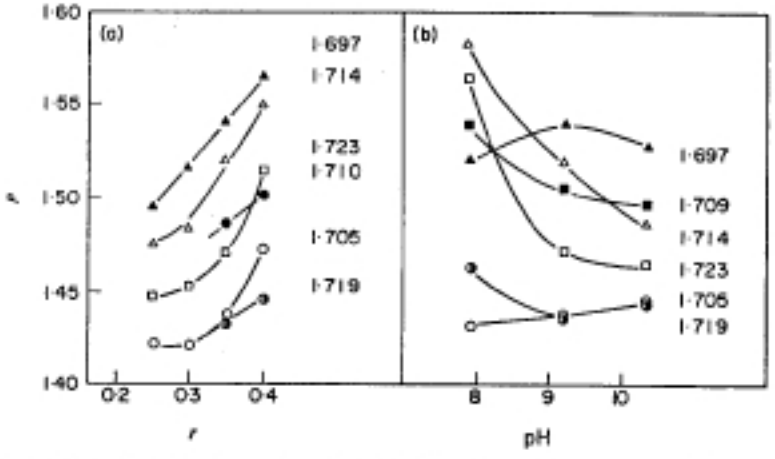

FIG. 6. Relations between the buoyant densities of calf liver DNA components in Cs_2SO_4–Ag^+ density gradients and (a) the ratio r, at pH 9·2; (b) the pH value, with the ratio r = 0·35. Data from Fig. 5.

(c) *Chemical and physical properties of the calf DNA components*

The chemical and physical properties of the DNA components described in the previous sections are given in Table 1. They were investigated on the central fractions of the densitograms shown in Figure 3(b) (hatched areas) except for the 1·705 and 1·723 g/cm³ components, which were derived from the experiment shown in Figure 1.

(i) *Buoyant densities*

(a) The three fractions from the DNA component (1·697 g/cm³) had identical buoyant densities (Fig. 3(c)). The σ values measured were close to 2·5 to 3 mg/cm³ for the 1·697 and the 1·704 g/cm³ components and close to 1·5 to 2 mg/cm³ for the 1·705, 1·710, 1·714 and 1·723 g/cm³ components. The σ values for bacterial DNAs having the same molecular weight are equal to 2 to 2·5 mg/cm³. (b) In alkaline CsCl, all components showed a single, symmetrical band except for the 1·714 g/cm³ component which displayed two bands (Fig. 7). (c) After alkali treatment and neutralization, the 1·697 and 1·704 g/cm³ components showed a buoyant density, 17 to 18 mg/cm³ higher than in the native state; in contrast, the 1·705, 1·709, 1·714 and 1·723 g/cm³ components showed a significantly lower increase, 9 to 13 mg/cm³, indicating that some renaturation had occurred. (d) After heating and reannealing, the 1·710, 1·714 and 1·723 g/cm³ components recovered their native buoyant densities, whereas the 1·709, 1·705 and 1·704 g/cm³ components showed densities higher by 3, 5 and 10 mg/cm³, respectively, than in the native state; the 1·697 g/cm³ was 12 mg/cm³ heavier (Fig. 8). The renatured 1·709 g/cm³ component showed some heavier material and a heavy shoulder which might correspond to a contaminating 1·714 g/cm³ component (see also subsection (ii); a very small amount of lighter material (1·699 g/cm³) was also found. Also shown in Figure 8 is the pattern of reannealed total calf thymus DNA, which is characterized by a light peak having a density of 1·709 g/cm³ and corresponding therefore to the main DNA component (see above), and a heavier peak tailing on the heavy side and having a 1·715 g/cm³ density, which probably comprises most of the other components (1·704, 1·709 and 1·714 g/cm³).

TABLE 1

Chemical and physical properties of calf DNA components[a]

Buoyant densities in CsCl												
Native DNA	Denatured DNA				Reannealed DNA		Melting temperature[b]		H.S.[b] %	$s_{20,w}$	G + C %	Amount in DNA (%)[f]
	Alkaline		Neutral									
ρ	ρ	$\Delta\rho$	ρ	$\Delta\rho$	ρ	$\Delta\rho$	T_m	σ				
1·697 L	—	—	—	—	—	—	—	—	—	23·8	39·2	} 50
1·697 M	1·743	46	1·714	17	—	—	67·8	2·9	38	23·3	38·7	
1·697 R	1·747	50	1·715	18	1·709	12	67·6	3·2	38	23·7	39·5	
1·704	1·745	41	1·721	17	1·714	10	70·5	3·3	33	24·8	47·6	25
1·705	1·752	47	1·716	11	1·710	5	73·4	0·9	31	18·7	48·9[e]	4
1·709	1·757	48	1·724	15	1·712	3	74·0[d]	3·7	33	19·1	54·2[e]	10
1·710	1·755	45	—	—	1·710	0	74·5	0·8	37	20·7	54·2	1·5
1·714	1·756 1·773	42 59	1·727	13	1·715	1	77·9	0·8	28	24·6	58·6	7
1·723	1·770	47	1·732	9	1·723	0	80·8	0·8	25	20·2	67·1	1·5
Total					1·709 1·715[c]					22·6	44·5[e]	100

[a] Buoyant densities in CsCl are used to indicate DNA components (see Discussion). For the origin of the samples see text. L, M and R are the 3 fractions of the main DNA component (see Results, section (c) (iv)).

[b] The standard deviation, 2σ, was measured between 20 and 80% of the total hyperchromicity. The σ values of bacterial DNAs are close to 2 to 2·5°C. H.S. is hyperchromic shift.

[c] As estimated from the data of Fig. 2. Minor components formed about 1% of total DNA.

[d] Average value, neglecting the discontinuity shown by this component.

[e] The 5-methylcytidine content could be estimated as lower than 1% in total DNA, and in the 1·705 and 1·709 g/cm³ components.

[f] See Fig. 8 and text.

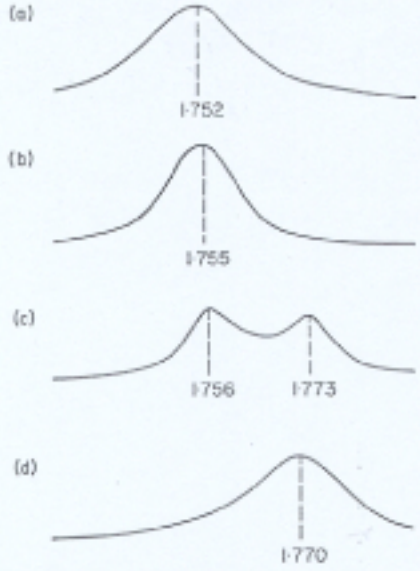

FIG. 7. Analytical alkaline CsCl densitograms of (a) 1·705, (b) 1·710, (c) 1·714, (d) 1·723 g/cm³ components. Expanded-scale scannings.

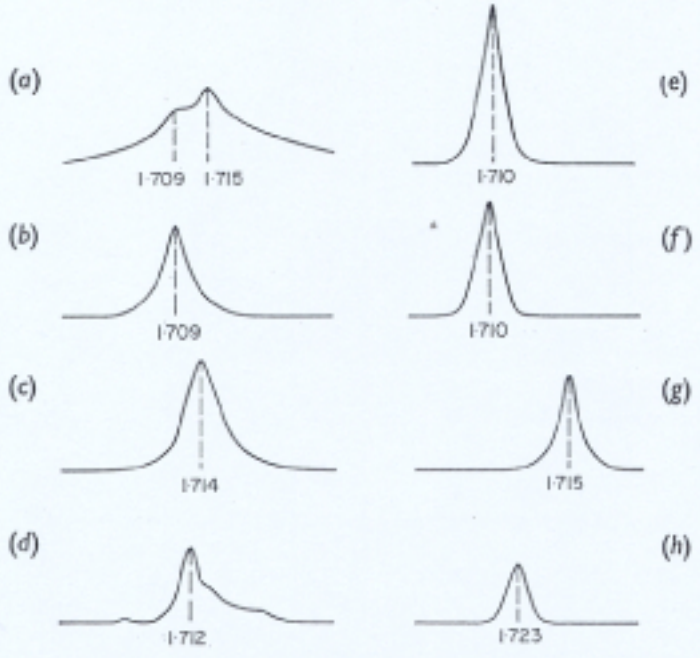

FIG. 8. Analytical CsCl densitograms of heated and reannealed total calf thymus DNA (a) and its components (b) 1·697, (c) 1·704, (d) 1·709, (e) 1·705, (f) 1·710, (g) 1·714 and (h) 1·723. Expanded-scale scannings.

(ii) *Absorbance–temperature curves*

Broad, symmetrical transitions were found for two fractions from the main DNA component (M and R) and by the 1·704 and 1·709 g/cm³ components (Fig. 9). The melting profile of the latter showed a discontinuity, possibly due to a contamination by the 1·714 g/cm³ component. The 1·719 g/cm³ component, as present in 1·705 g/cm³ component preparations, was found to have a very high melting temperature (83°C in 0·1 × SSC; not shown). Finally, the melting profiles of the 1·705, 1·710, 1·714 and 1·723 g/cm³ components were characterized by very sharp transitions.

The hyperchromicities of DNA components decreased with increasing G + C content, as expected (Felsenfeld & Hirschman, 1965), except for the 1·710 g/cm³ component.

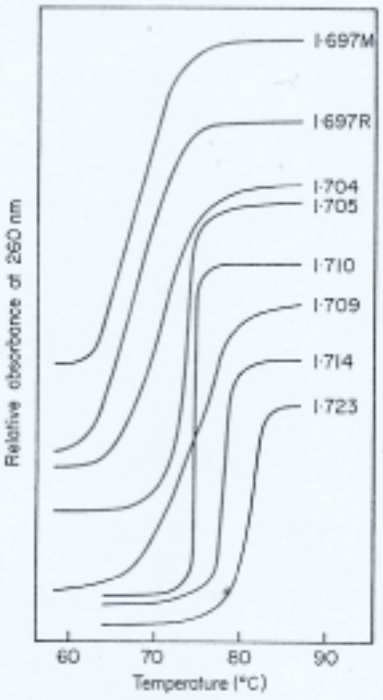

FIG. 9. Absorbance–temperature profiles for calf thymus DNA components in 0·1 × SSC.

(ii) *Sedimentation coefficients*

Those of DNA components ranged from 19 to 24 S. It is likely that the differences found reflect a preferential enzymic degradation of some components during DNA extraction.

(iv) *Base composition*

The G + C contents of the three fractions (L, M, R; Fig. 3(b) and (c)) of the main DNA component were found to be identical and equal to 39%. The 1·704 g/cm³ component exhibited a G + C level of about 48% compared with the other components, which ranged from 49 to 67%. Base pairing was within 1%.

(v) *Relation between G + C contents, buoyant densities and melting temperatures*

Figure 10 shows that the buoyant densities in CsCl of all DNA components are lower than those expected from their G + C contents, on the basis of the relation of Schildkraut *et al.* (1962), the deviation being, however, minimal and of doubtful significance for the main DNA component. The melting temperatures showed deviations from the relationship of Mandel *et al.* (1970) only in the case of the 1·704 and 1·709 g/cm^3 components; much smaller deviations were found for the main band DNA and the 1·723 g/cm^3 component. If corrected for the low extent (less than 1%) of methylation that was found in total DNA and in some of the DNA components, buoyant densities should be increased by, at most, 1 mg/cm^3 and T_m values should be very slightly decreased, since methylation causes a decrease in buoyant density (Kirk, 1967) and an increase in T_m (Dawid *et al.*, 1970). These corrections are far from sufficient to eliminate the deviations observed and, in fact, would cause the T_m values fitting the Mandel *et al.* (1970) relation to deviate. In addition, the deviations observed in buoyant density and T_m occur in the same direction in the case of the DNA components considered here.

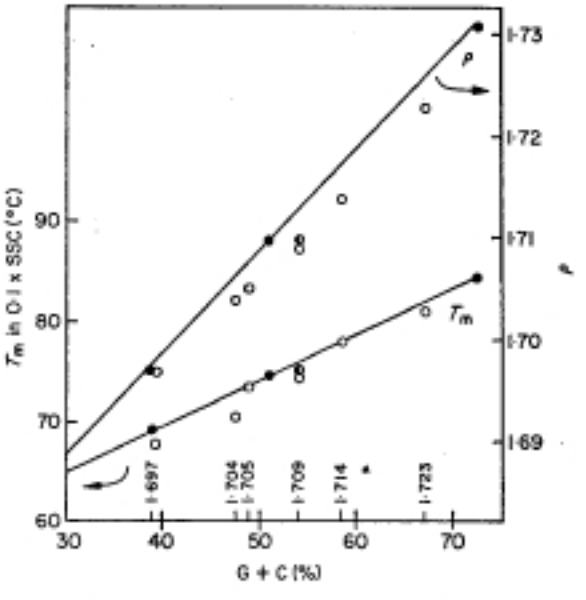

FIG. 10. Plots of buoyant density and melting temperature of calf thymus DNA components against their G + C contents (○); (◐) the 1·710 g/cm^3 component. (●) Experimental values obtained with *H. influenzae*, *E. coli* and *Micrococcus luteus* (*M. lysodeikticus*) in our laboratory. Straight lines show the relations established for bacterial DNAs by Mandel *et al.*, 1970.

4. Discussion

(a) *DNA components of the bovine genome*

Under the experimental conditions used in our fractionations, preparative Cs_2SO_4–Ag^+ density gradient centrifugation permits the resolution of calf DNA "molecules" having molecular weights of 5 to 7×10^6 into a number of discrete components. For the sake of the discussion, we will divide these components into three groups.

(i) The 1·697, 1·704 and 1·709 g/cm³ components represent about 50, 25 and 10% of the genome, respectively, the estimates given for the first two components possibly being wrong by ±5%. The three components are characterized by an *intermolecular* compositional homogeneity†, as indicated by the fact that the bands formed in CsCl density gradients are unimodal and symmetrical, and have a standard deviation practically identical to those exhibited by *Escherichia coli* and *Hemophilus influenzae* DNA samples of equal molecular weight; such a band symmetry persists in alkaline CsCl density gradients, and after heat-denaturation and reannealing. The unimodal yet broad thermal transitions of the three components are related, therefore, to *intramolecular* compositional heterogeneity.

The melting–reannealing experiments showed that: (1) the main DNA component exhibited a slight yet significant extent of renaturation, its buoyant density being only 12 mg/cm³ higher than in the native state, as opposed to a difference of 17 to 18 mg/cm³ after alkylation followed by neutralization; this behaviour explains the appearance of the main DNA component as a shoulder of density 1·709 g/cm³ on heating and reannealing total calf DNA (Fig. 8). (2) The tendency to renature upon reannealing is more pronounced in the 1·704 and, particularly so, in the 1·709 g/cm³ component, the buoyant density of the latter almost reaching the native value.

(ii) The components having buoyant densities of 1·705, 1·710, 1·714 and 1·723 g/cm³ represent 4, 1·5, 7 and 1·5% of the genome, respectively, and are characterized by the sharp melting behaviour typical of "satellite" DNAs. Except for the 1·710 g/cm³ component, which is reported here for the first time, they had been previously identified and characterized to some extent. Table 2 shows a comparison of our present results with those reported by other authors. Regarding the 1·723 g/cm³ component, no strand separation was observed in alkaline CsCl, in contrast with the data of Corneo *et al.* (1970), and in agreement with previous results from our laboratory (André, 1971). Our results strongly confirm the idea that strand separation in alkali cannot be considered to be a general property of satellite DNAs (Walker *et al.*, 1969). On renaturation, the 1·710, 1·714 and 1·723 g/cm³ components recovered their native buoyant density, whereas the 1·705 g/cm³ component showed a higher density, i.e. 1·710 g/cm³.

(iii) A number of other components were not characterized, but were identified. These comprise the 1·719 g/cm³ component, and additional components present in minute amounts on the heavy side of the densitogram (1·697, 1·698 and 1·714 g/cm³) and in fractions 12 to 17 of Figure 2 (1·699 and 1·716 g/cm³). In spite of the fact that in most cases these components only formed much less than 1% of total DNA, they were easily identified on the basis of (1) their buoyant densities in CsCl; (2) their positions in Cs_2SO_4–Ag^+ density gradients; (3) their shifts in density in these gradients caused by changes in *r* and pH values. For instance, the minor 1·719 g/cm³ component bands, at pH 9·2 and 10·3, at the position of the 1·705 g/cm³ component; being close to the 1·723 g/cm³ component, it might be confused with the latter; when centrifuged at pH 7·9, however, the 1·719 g/cm³ component is only slightly shifted to a heavier position, while the 1·723 g/cm³ component moves to the heavy side of the main

† Allowance being made for phenomena associated with the slight contamination of 1·714 g/cm³ component in the 1·709 g/cm³ component (the discontinuity in the melting curve and the multimodal pattern of the reannealed component in CsCl).

TABLE 2

Data of the 1·705, 1·710, 1·714 and 1·723 g/cm³ components

Component	%	Buoyant densities in CsCl			Nucleoside analysis	T_m
		Native DNA	Denatured DNA Alkaline	Neutral		
1·605†	4	1·705	1·752	1·716	48·9	73·4
‡	3	1·706	1·770		46·0	73·0
1·710†	1·5	1·710	1·755	—	54·2	74·5
1·714†	7	1·714	1·756 1·773	1·727	58·6	77·9
‡	7	1·713	1·773 1·783		55·0	76·0
§	10	1·713	1·750 1·769	1·728		76·7‖
1·723†	1·5	1·723	1·770	1·732	67·1	80·8
§	2	1·721	1·768 1·773	1·733		

† Present work.
‡ From Yasmineh & Yunis (1971).
§ From Corneo *et al.* (1970).
‖ Recalculated from data obtained in SSC by Polli *et al.* (1966).

band; similarly, the effect of r value changes on the banding positions of the two components is quite different (Fig. 6(a)).

(b) *Comments on the DNA components*

(i) No component that could not be easily identified with those observed in the experiment shown in Figure 2, was revealed by experiments involving: (1) Cs_2SO_4–Ag^+ density gradient centrifugation at different pH and/or r values; (2) hydroxyapatite chromatography; and (3) CsCl density gradient centrifugation in the presence of actimomycin (unpublished results). Without completely ruling out the possibility that some components escaped our analysis, we tend to think that the picture given by the present data defines to a very great extent the DNA components present in calf DNA at the molecular weight level investigated. It should be stressed that DNA components present at levels between 1 and 0·1% can still be recognized by the type of analysis used here.

(ii) It has already been stressed that the DNA components investigated here are those which can be separated at an average molecular weight of 5 to 7×10^6. It would be of obvious interest to know which components disappear in DNA preparations of higher molecular weight and which new components appear as a consequence of DNA degradation. We have not attempted to explore any of these alternative situations, since the preparation of well-deproteinized DNA of higher molecular weight is quite difficult and since the degradation of unfractionated DNA leads to Cs_2SO_4–Ag^+ densitograms that are difficult to interpret. We are presently investigating, however, the effects of controlled degradation on the separated 1·697, 1·704 and 1·709 g/cm³ components.

(iii) As expected, no difference was found between the calf thymus and the calf liver DNA preparations in either the quality and/or the quantity of the components, in spite of the very large number of different cases in which the component was made.

(iv) No specific function can unequivocally be associated at the present time with any of the DNA components described. Three possible identifications deserve, however, to be mentioned here: (1) On the basis of the results of Sinclair & Brown (1971), who showed calf ribosomal cistrons to be associated with a DNA of buoyant density equal to 1·719 g/cm^3, our 1·719 g/cm^3 component might correspond to the ribosomal cistrons. It should be noted that the 1·719 g/cm^3 component shares with the ribosomal cistrons of *Xenopus laevis* (Dawid *et al.*, 1970) the property of being the lightest component in Cs_2SO_4–Ag^+ density gradients. (2) The position of mitochondrial DNA in the Cs_2SO_4–Ag^+ density gradient has also not been established; unless buried in the main band, mitochondrial DNA (which has a buoyant density of 1·699 g/cm^3 in vertebrates, and represents less than 1% of the total DNA, Borst & Kroon, 1969), might correspond to one of the minor 1·697 to 1·699 g/cm^3 components seen in the experiment shown in Figure 2. The 1·699 g/cm^3 component banding at a position close to the 1·709 g/cm^3 component (Fig. 2) is particularly suspect, since a small 1·699 g/cm^3 band has been seen in renatured 1·709 g/cm^3 component (Fig. 8). (3) The minute 1·714 g/cm^3 component banding at a very heavy position in the Cs_2SO_4–Ag^+ density gradient (Fig. 2) might correspond to the "single-stranded" DNA fraction found by Tapiero *et al.* (1972) in Chinese hamster nuclei, since it exhibits both a high density in CsCl and the strong Ag^+ binding of single-stranded DNA (Jensen & Davidson, 1966). (4) The possibility should be kept in mind that one or more of the minute DNA components be of viral or bacterial origin.

(v) No renaturation kinetics studies have been carried out so far on any of the DNA components isolated in the present work. It is conceivable, however, that the sharp melting components correspond to the fast-renaturing fraction of Britten & Kohne (1968), whereas the 1·709, the 1·704 and the 1·697 g/cm^3 components might give rise to DNA fragments having the renaturation behaviour of the intermediate and/or slow fractions. Investigations, currently under way on the intramolecular heterogeneity of these components, should yield interesting results. Renaturation kinetics studies on fractionated DNA fragments originating from these components should also be interesting.

(vi) The chromosomal localization of the DNA components has not been investigated so far. The results of Yasmineh & Yunis (1971) have shown, however, that the 1·705 and 1·714 g/cm^3 components are associated with heterochromatin.

(vii) A number of "anomalous" physical properties have been found for many of the DNA components investigated here. The buoyant densities are all too low compared to bacterial DNAs of identical G+C content, except for the main DNA component. The melting temperatures, the silver binding and the elution molarity from hydroxyapatite are anomalous for several of the components. Since the presence of rare bases cannot fully account for these phenomena, it is conceivable that they are rather related to structural features associated with repetitive nucleotide sequences. This explanation, already suggested by Corneo *et al.* (1968) for the satellite DNAs from

mouse and guinea pig and by Bernardi *et al.* (1972) for yeast mitochondrial DNAs seems to apply not only to typical satellite DNAs like the 1·705, 1·710 and 1·723 g cm^3 components, but also to the 1·704 and 1·709 g/cm^3 components. It is interesting that such anomalous properties have not been found for the main DNA component.

(c) *Terminology*

We have purposely refrained from using the term satellite for indicating the DNA components isolated and characterized in the present work. According to the original definition (Kit, 1961), this term would only apply to the 1·714 and 1·723 g/cm^3 components, which are the only ones to show up in CsCl density gradients of calf DNA. According to the definition of Walker (1971), the term satellite would indicate "a native fraction of the chromosomal DNA, which after isolation by any method gives a narrow, unimodal band in CsCl because of common properties shared by its sequences". It is evident that while the first definition is too restrictive, since it separates the 1·705 and 1·710 g/cm^3 components (which can only be isolated by Cs_2SO_4–Ag^+ density gradients) from the similar 1·714 and 1·723 g/cm^3 components, the second definition is too loose because it applies to all DNA components investigated in the present work, regardless of the very different properties they have.

We have preferred to indicate the DNA components by their native buoyant density in CsCl rather than by Roman numerals or Greek letters, for three reasons: (1) the indication is more informative; (2) buoyant densities in CsCl are reproducible within ± 1 mg/cm^3 in different laboratories; (3) even in cases, like the present one, in which the number of components dealt with is large, buoyant densities lend themselves to less confusion than other indications.

5. Conclusion

The main conclusion from this work is that, at a size level of about 5 to 7×10^6 molecular weight, the bovine genome mainly consists of seven DNA components (Fig. 11) that are formed by different populations of DNA molecules essentially homogeneous in their physical and chemical properties. Four of these components

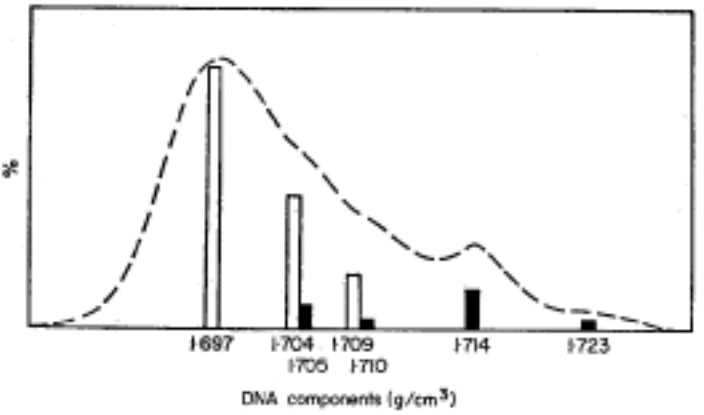

FIG. 11. A histogram of the DNA components of the bovine genome. The height of the bar is proportional to the percentage of each component; filled bars correspond to the sharp melting, empty bars to the broad melting components. The broken line is an enlarged band pattern in CsCl density gradient of total calf DNA.

(1·705, 1·710, 1·714 and 1·723 g/cm³), forming together about 14% of the genome, are characterized by the high intramolecular compositional homogeneity typical of satellite DNAs and do not deserve any additional comments.

The demonstration of three well-defined components (1·697, 1·704 and 1·709 g/cm³), forming 85% of the genome, sharing an evident intramolecular heterogeneity but endowed with distinct properties, is a new important finding, since it is at variance with the widespread view that the bulk of the genome of higher organisms is formed by DNA molecules showing a continuous variation in their G + C content. Such a view is apparently supported by base analysis of fractions of mammalian DNA bands in preparative CsCl density gradients (Tapiero *et al.*, 1972). In fact, such results do not contradict our conclusions, since they can be explained by the very small number of fractions analysed and by the low resolution of preparative CsCl density gradients, which leads to the formation of bands in which a series of components of increasing density largely overlap each other. In contrast, under comparable DNA concentration and centrifugation conditions we find that calf DNA bands over a volume 2·5 times larger in Cs_2SO_4–Ag^+ than in CsCl preparative gradients.

In contrast with the satellite components, which are known to be quite different even in closely related species (Walker, 1971), the components accounting for the "main-band" DNA may have a wider relevance. It is well known that mammalian DNAs are very close in over-all base composition (Sueoka, 1961), in doublet frequency (Swartz *et al.*, 1962) and in the frequency patterns of short nucleotide sequences, after digestion by DNases (S. D. Ehrlich & G. Bernardi, unpublished data). On the other hand, their CsCl bands show almost identical buoyant densities (Arrighi *et al.*, 1970) and a distortion on the heavy side that is not at all, or not exclusively, due to the presence of satellite components (unpublished results). It is worth considering, therefore, that mammalian DNAs generally contain a main DNA component similar to that found here, and probably also components similar to the 1·704 and 1·709 g/cm³ components of calf DNA.

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