

ISOPYCNIC CENTRIFUGATION OF CHROMATIN IN RENOGRAFIN SOLUTIONS

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ABSTRACT

Solutions of Renografin (30–60%) can be centrifuged to form density gradients in the range from 1.0 g/cm³ to 1.4 g/cm³, or, alternatively, preformed gradients can be made which under appropriate conditions of centrifugation have an indefinite stability. Such solutions have a low viscosity and a relatively low ionic strength. The density of DNA in such solutions is surprisingly low (~1.14 g/cm³). Crude chromatin can be sedimented to an equilibrium position in such gradients, corresponding to a density of 1.24 g/cm³, or slightly lower, depending on the method of preparation. The complex is shown to contain DNA, RNA, protein, and possibly some lipoprotein. Most of the RNA can be removed with RNase without any significant effect on the density of the chromatin.

INTRODUCTION

A number of methods are available for the isolation and study of nucleic acid-protein complexes (see von Hippel and McGhee, 1972, for a review). Isopycnic centrifugation in a stable, self-generated gradient has not been widely used, because most nucleic acid-protein complexes are dissociated at the high salt concentrations of, for example, cesium chloride gradients. Some success in attempts to circumvent this problem has been achieved by cross-linking the complexes with formaldehyde (see for example Georgiev and Samarina, 1971), but it might be desirable to reduce manipulations and treatments to a minimum before centrifugation. Furthermore, there is the problem of irreversible changes being introduced, precluding further analysis of the proteins in their native forms. Other experiments have tried to avoid the high salt concentrations by the use of nonionic solutes. Raynaud and Ohlenbusch (1972) have described the use of gradients of sucrose and glucose, and Hossainy, Zweidler, and Block (1973) have reported results with chloral hydrate gradi-

ents. We describe below our initial experiments with gradients formed by Renografin.

Renografin gradients have been in use for the separation of sporulating and nonsporulating bacteria (Tamir and Gilvarg, 1966), for the fractionation of the components of bacterial flagellae (Dimmitt and Simon, 1971), for the fractionation of a population of yeast cells according to cell age (Hartwell, 1970), and in one series of reports efforts have been described to separate bacterial DNA from DNA attached to a membrane (Ivarie and Pene, 1970 and 1973). A separation of DNA from covalently linked DNA-Ficoll copolymers has also been reported (Scheffler and Richardson, 1972).

MATERIALS AND METHODS

Cells and Nuclei

Chinese hamster fibroblasts were grown in minimum essential Eagle's medium (MEM) as described by Scheffler and Buttin (1973). After labeling, the cells were washed three times with cold TD buffer (per liter: 8 g NaCl, 0.38 g KCl, 0.25 g Na₂HPO₄ · 12 H₂O, 3.0 g Tris,

pH 7.4), then scraped from the plates using a rubber policeman with cold TD. They were collected by centrifugation and resuspended in a small volume of TD ($\sim 10^7$ cells/ml), an equal volume of 1% Nonidet NP40 in TD was added, and the cells were left on ice for 60 min. The resulting suspension of nuclei was layered on top of a 0.5-M sucrose solution in TD buffer, and the nuclei were collected as a pellet after a brief centrifugation. They were resuspended in 10–20 times their packed volume of TD buffer.

A crude extract was prepared by either sonicating the suspension by several bursts from a sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) equipped with a microtip, or alternatively, by homogenizing the suspension in an Elsevier homogenizer with a tight-fitting, motor-driven Teflon pestle (Bellco Glass, Inc., Vineland, N. J.). The homogenized or sonicated samples were centrifuged immediately after their preparation (see below). We also prepared chromatin by the procedures of Bhorjee and Pederson (1973), and of Bonner et al. (1968).

Labeling of Cells

All radioisotopes were obtained from New England Nuclear, Boston, Mass. The DNA was labeled during incubation with tritiated thymidine or [^{14}C]thymidine. Nuclear RNA was labeled with tritiated uridine during a short exposure (~ 3 h), protein was labeled in different experiments with either [^3H]leucine, [^{14}C]- or DL-[^3H]lysine, or [^3H]phenylalanine, and the membranes were labeled with either [^{14}C]choline or [^3H]fucose (Bhorjee and Pederson, 1973). The specific activities and labeling times are given in the figure legends. In each case the medium was MEM. T4 DNA labeled with [^{14}C]thymidine was prepared by phenol extraction of T4 phage prepared by standard methods.

Gradients and Centrifugation

Renografin, which was obtained as a 76% solution from E. R. Squibb & Sons, Inc., New York, also contains 0.32% sodium citrate, 0.04% disodium edetate (EDTA), and 0.04 meq per cm^3 sodium ion. The 76% Renografin is made up of 10% of the sodium salt and 66% of the methylglucamine salt of 3,5-diacetamido-2,4,6-triiodobenzoic acid. Further dilutions of this material are described in the figure legends. To make preformed gradients, we used a gradient maker from Hoefer Scientific Instruments, San Francisco, Calif., in conjunction with a peristaltic pump (Cole-Parmer Instrument Co., Chicago, Ill.), and the gradients were made by displacement from the bottom of the lighter by the heavier fluid.

Polyallomer tubes containing 4.5 ml of liquid were centrifuged in an SW 50.1 rotor in a Beckman centrifuge (model L-2, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Temperature, rotor speed, and duration of the run are given in the figure legends. The gradients were dripped and collected with a fraction

collector from Hoefer Scientific Instruments. The fractions were mixed with scintillation fluid containing toluene and Triton (Ihler and Rupp, 1966), and counted in a Beckman scintillation counter (model LS-230, Beckman Instruments, Inc., Fullerton, Calif.).

The refractive index of Renografin solutions was measured with a Zeiss refractometer, (Carl Zeiss, Inc., New York), and the correlation between refractive index and density was established by weighing 100- μl samples of solutions of known refractive index (see Results).

Ribonuclease A (from Worthington Biochemical Corp., Freehold, N. J.), was treated at 80°C for 10 min at a concentration of 2 mg/ml in $1 \times \text{SSC}$ (0.15 M NaCl, 0.015 M sodium citrate), pH 5.0.

RESULTS

Self-Generated Gradients

Fig. 1 A is a plot of the refractive index (R) vs. the density (ρ) of Renografin solutions measured at room temperature. The graph can be represented by the analytical expression $R = 0.272_7 \rho + 1.062$. Uniform solutions with initial densities of 1.17_6 g/cm^3 (30%), or 1.23_7 g/cm^3 (45%) form gradients shown in Fig. 1 B after 160 h of centrifugation at 25,000 rpm. The denser solution may not have reached equilibrium after this time. We then explored the banding of pure DNA in such gradients and found that in a gradient formed from a 60% Renografin solution ($\rho_0 = 1.33_7 \text{ g/cm}^3$) the DNA ended up floating on top; it barely entered the gradient formed by a 45% solution (1.23_7 g/cm^3), while a sharp band was formed in a gradient formed by a 30% solution

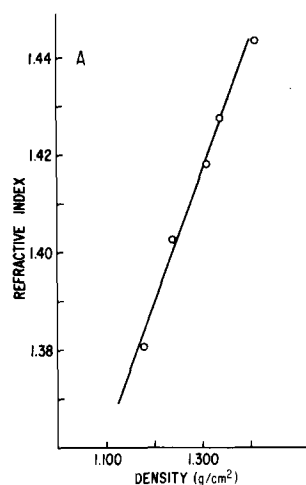


FIGURE 1 A The refractive index of Renografin solutions as a function of their density. Measurements were made as described under Materials and Methods.

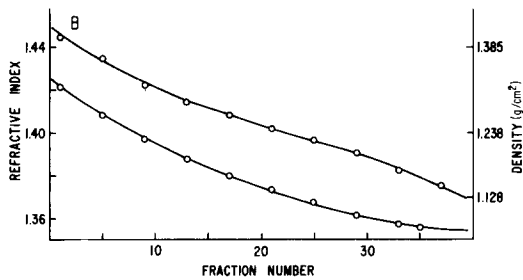


FIGURE 1 B Equilibrium density gradients formed after centrifuging 30% and 45% solutions of Renografin ($\rho_0 = 1.176 \text{ g/cm}^3$ and 1.237 g/cm^3 , respectively) for 160 h at 25,000 rpm and at 18°C in an SW50.1 rotor; 4.5 ml of solution were overlaid by a few drops of paraffin oil in polyallomer tubes. Six drops were collected per fraction and the refractive index was determined with a Zeiss refractometer.

(1.176 g/cm^3) (Fig. 2). This band is formed regardless of whether the DNA is layered on top or whether it is initially uniformly distributed throughout the tube, indicative of a true equilibrium's being approached (see below for further discussion).

Initial experiments (not shown) had indicated that λ bacteriophage particles banded at a density significantly higher than that of DNA, and from a consideration of the relatively low ionic strength of a 30% Renografin solution it became of interest to investigate the stability and density of DNA-protein complexes in such gradients. A crude, sonicated preparation of tritium-labeled chromatin was prepared as described under Materials and Methods, and either mixed with a 30% Renografin solution, or layered on top of a tube filled with such a solution. Fig. 2 also shows an example of such an experiment. The run length was 72 h.

TIME REQUIRED TO REACH EQUILIBRIUM: The position of the chromatin and DNA bands after a 72-h run were quite reproducible, but in order to get a better measure of the required running time, we interrupted the centrifugation after various periods of time. One gradient was collected for scintillation counting while another was collected for a determination of the refractive index and density throughout the gradient. The results are shown in Fig. 3 A and B. It is clear that the gradient is established slowly, and although a chromatin band is already visible after 24 h, its final position may not have been reached even after 72 h. Thus, although a useful separation may have been achieved after 72 h, we have not quite reached a true equilibrium.

In other experiments we reduced the length of the liquid column in the tube by half, but even then a change in the refractive index gradient was observed between 48 and 72 h of running time. Under these conditions, as expected, the gradient was steeper and the separation of the DNA and chromatin bands was considerably reduced.

THE RATE OF DISSOCIATION OF THE DNA-PROTEIN COMPLEX: If the dissociation of protein from chromatin is slow but appreciable over the time-periods involved, one might also expect a slow drift of the DNA peak position towards lighter densities. To investigate this possibility we rebanded the material from the peak fractions in another 72-h run under the same conditions, i.e., in a 4.5-ml gradient. The DNA from the chromatin band now banded at a density lower than that of the T4 marker DNA (Fig. 4), indicating that probably complete dissociation of the protein-DNA had occurred. The difference in density between T4 and hamster DNA may be due to the glucosylation of the T4 DNA.

It was also observed that if T4 [^{14}C]DNA was mixed with the sonicated nuclear extract, a fraction of it banded with the chromatin or in fact at a slightly higher density (Fig. 5). This fraction was variable but never negligible, depending on whether the T4 DNA was layered on the 30% Renografin solution containing the chromatin, or mixed with the sonicated extract before being diluted by the Renografin solution. Surprisingly,

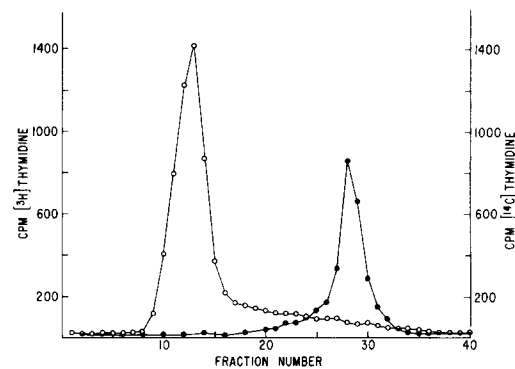


FIGURE 2 $10 \mu\text{l}$ of T4 [^{14}C]DNA were mixed with 4.5 ml of a 30% Renografin solution and spun at 25,000 rpm for 72 h at 18°C in an SW50.1 rotor. Fractions (six drops) were collected and counted as described under Materials and Methods. In a separate centrifuge tube $100 \mu\text{l}$ of sonicated hamster nuclei labeled for 6 h with [^3H]thymidine were centrifuged identically. (^3H]thymidine: specific activity 20 Ci/mmol , $1 \mu\text{Ci/ml}$). \circ , hamster chromatin; \bullet , T4 DNA.

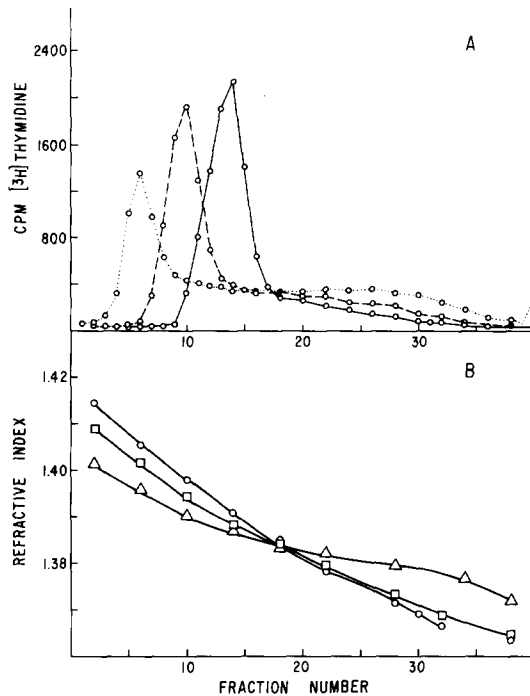


FIGURE 3 A Material from [^3H]thymidine-labeled, sonicated nuclei was centrifuged as described in Fig. 2, but the centrifuge was stopped at 24, 44, and 72 h to collect one gradient for counting (\circ ·····, \circ — —, \circ — —, respectively).

FIGURE 3 B 30% Renografin solutions were centrifuged in the SW50.1 rotor at 25,000 rpm at 18°C, and the centrifuge was arrested at various times to collect fractions from one tube for a refractive index determination: Δ , 39 h; \square , 65 h; \circ , 88 h.

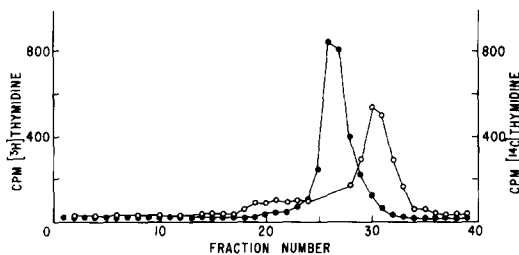


FIGURE 4 The peak fractions containing [^3H]thymidine-labeled DNA from a run such as shown in Fig. 2 were pooled and mixed again with fresh 30% Renografin solution; 10 μl of T4 [^{14}C]DNA were layered on top, and the centrifugation was carried out as before for 72 h. \bullet , T4 [^{14}C]DNA; \circ , [^3H]DNA from hamster nuclei.

no complexes of intermediate density were formed, i.e., the complexing of T4 DNA was an all or nothing phenomenon. In this case it was again observed that the complexed T4 DNA banded at a

heavier density than the hamster chromatin complex.

THE POSITION OF PROTEIN ON THE GRADIENT: In order to obtain some information about the distribution of nuclear proteins on such a gradient, we isolated and sonicated nuclei after labeling the cells with tritiated leucine for 1 day, and the results are shown in Fig. 6. Most of the protein counts appear to be pelleted and they are recovered in the centrifuge tube. A peak is observed on a broad shoulder extending from the bottom to the top of the tube and the peak position corresponds to the position of the DNA band. In the presence of detergent we see only the broad shoulder while the DNA moves to the top of the gradient.

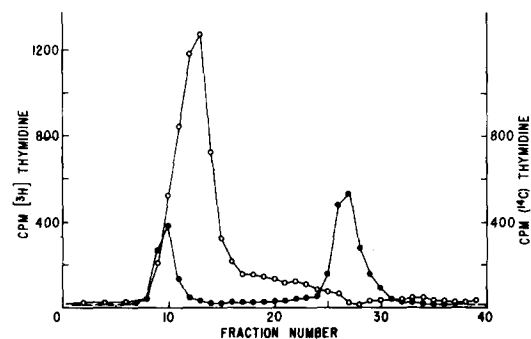


FIGURE 5 A crude, sonicated extract of hamster nuclei labeled with [^3H]thymidine was mixed with Renografin solution, and purified T4 DNA was layered on top of the tube at the beginning of the run. The conditions were as described in Fig. 2. \bullet , T4 [^{14}C]DNA; \circ , [^3H]DNA in hamster chromatin.

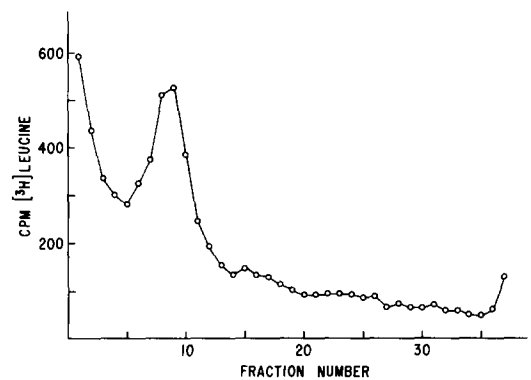


FIGURE 6 Hamster nuclei were isolated as described after a 24-h labeling period with [^3H]leucine, specific activity 47 Ci/mmol, 10 $\mu\text{Ci}/\text{ml}$. After sonication, the crude extract (100 μl) was mixed with 4.5 ml of a 30% Renografin solution and then centrifuged for 72 h at 25,000 rpm at 18°C.

Centrifugation in Preformed Gradients

It appeared that although usable gradients of Renografin could be generated by centrifugation, the time required to reach equilibrium was rather long, and we therefore attempted to establish conditions under which preformed gradients would be relatively stable. A preformed gradient with the range 30–60% (1.17₆–1.33₇ g/cm³) was changed relatively little over 22, 46, and 70 h of running time. In such a gradient pure T4 DNA and purified RNA from *Escherichia coli* band or even float near the top of the tube.

Under the same conditions, labeled DNA from homogenized or sonicated nuclei is found in a band at a density of 1.235 ± 0.005 g/cm³. These results are shown in Fig. 7 A–D: material from homogenized nuclei has reached its equilibrium position already after 24 h of centrifugation, with only a small change (within experimental uncertainty) observed upon longer centrifugation; material from sonicated nuclei requires a longer time to reach equilibrium, although after 46 h the changes are hardly significant (Fig. 7 C and D). In the latter case the DNA peak is broader, probably due to the reduced size of the fragments and perhaps also as a result of heterogeneity of the complex with which DNA is associated. The main peak is again at the density of the homogenized material. The same density of 1.235 ± 0.005 g/cm³ is also observed if the crude material is banded in different preformed gradients. There is no evidence for intact nuclei at this position: the particular fractions are very viscous, and microscope observations have shown that nuclei lyse in Renografin solutions of similar concentrations.

We also attempted to reband material from homogenized nuclei isolated in the peak fractions. These were diluted fourfold with cold TD buffer, layered on a newly made gradient, and run for another 24 h. The result was that the material became distributed in two unequal fractions: about 25–30% of the counts ([¹⁴C]Tdr in DNA) were found in a peak corresponding to a density of approximately 1.2 g/cm³, while the remainder was found floating very near the top of the gradient.

THE POSITION OF RNA IN THE GRADIENT: One of our aims had been to see if nuclear, heterogeneous RNA could be separated from the DNA by this procedure, but as already seen in Fig. 7, the RNA label is intimately associated with that in the DNA. That we are indeed observing RNA is proven by an experiment showing that more than

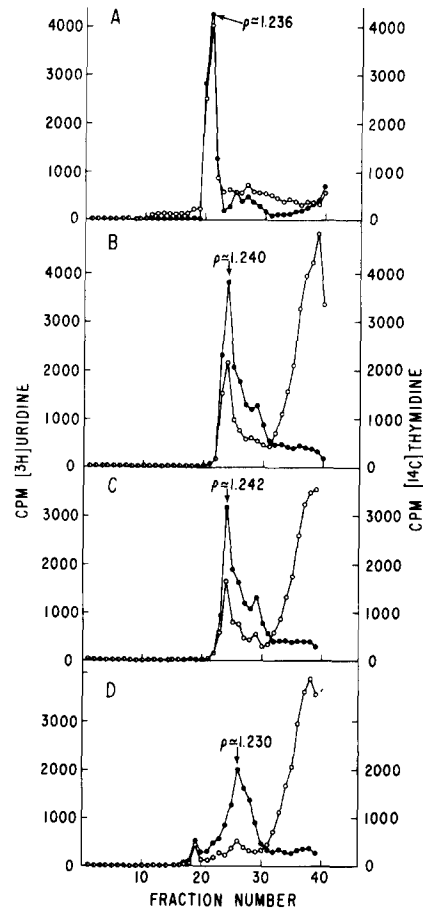


FIGURE 7 Centrifugation of homogenized and sonicated nuclear suspensions in preformed (Renografin) gradients (30–60%). Nuclei had been labeled with [¹⁴C]thymidine (●) (specific activity 51.5 mCi/mmol, 0.1 μCi/ml) for 12 h followed by a 4-h labeling with [³H]uridine (○) (specific activity 28 Ci/mmol, 4 μCi/ml). The homogenized material was run for 23 and 46 h (A and B), while the sonicated material was run for 46 and 70 h (C and D). Refractive index determinations were made on a few aliquots on either side of the peak and the density at the peak position was calculated by interpolation. Conditions of centrifugation: 25,000 rpm, 2°C.

90% of the [³H]uridine label is converted to acid-soluble counts by a 24-h incubation at room temperature in 0.2 M NaOH. Furthermore, conversion of [³H]uridine to thymidine should lead to a loss of the label, although conversion to cytosine without loss of label is a possibility.

In order to see whether the digestion of RNA by ribonuclease A had any effect on the position of DNA, nuclear homogenates labeled with

[¹⁴C]thymidine and [³H]uridine were incubated for various lengths of time at 0°C, or for 1 h at room temperature. These were then layered on the preformed gradient and centrifuged as before. The results are shown in Figure 8 A–D). In an untreated sample, most of the RNA is found in the same fractions as the DNA (Fig. 8 A). Incubation at 0°C releases a large fraction of the RNA as mono- and oligonucleotides which remain at the top of the gradient. Repeatedly, a shoulder of DNA is observed on the light side of the DNA band. When the incubation is carried out at room temperature, most of the RNA becomes converted

to floating material, while the DNA peak becomes a little broader and slightly shifts to lower densities, again barely outside of experimental uncertainty.

ASSOCIATION OF PROTEIN WITH THE DNA AND RNA: As already seen in Fig. 6, protein is associated with the DNA band and the bulk of the protein appears to be at a higher density at the end of a long run. We should also emphasize that these results were obtained with sonicated nuclear material which was mixed with a 30% Renografin solution and run for 72 h. With shorter running times and starting with homogenized material layered on top of a preformed gradient, the proteins not involved in large complexes with DNA or RNA are not expected to reach their equilibrium position. Some typical results are presented in Fig. 9 A and B. With labeled phenylalanine or lysine as precursors, counts are observed in the gradient at positions denser than the DNA complex, at the position of the complex, and near the top of the gradient. It seems reasonable, then, to conclude that proteins are at least in part responsible for the density shift experienced by the DNA.

CHROMATIN PREPARED BY OTHER TECHNIQUES: Our preparation of chromatin is a very crude one and we therefore wished to apply our banding technique to chromatin isolated by conventional procedures. Fig. 10 A shows the banding of chromatin isolated by the procedure of Bonner et al. (1968) after 40 h of centrifugation on a preformed gradient (30–60% Renografin). The density at the peak position corresponds to 1.20 g/cm³. The peak did not shift after an additional 24 h of centrifugation. Very similar results were obtained with chromatin isolated by the method of Bhorjee and Pederson (1973). Fig. 10 B and C shows our results with that material after 41 and 91 h of running time. The density at the peak is again 1.20 g/cm³, but this time we see a shoulder on the light side of the peak, while a shoulder on the heavy side was seen with Bonner chromatin. Also shown in Fig. 10 B and C is the position of hamster DNA obtained after repeated phenol extraction of the chromatin, and the arrow in the figure shows the position of pure T4 DNA. The purified DNAs were run simultaneously in separate tubes. It is clear that there is a significant density shift relative to pure DNA but the density is not as great as that observed for our crude preparation.

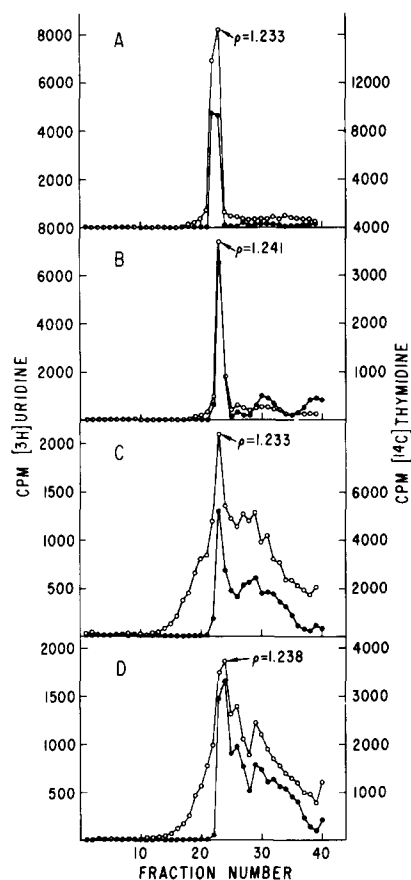


FIGURE 8 Digestion of the nuclear RNA by ribonuclease A. Nuclei labeled as described in the previous figure were homogenized and then treated with 0.7 mg/ml of ribonuclease prepared as described in Materials and Methods. (A) control, no ribonuclease treatment; (B and C) ribonuclease treatment for 1 or 2 h at 0°C; (D) ribonuclease treatment for 1 h at room temperature. Centrifugation and density determinations were made as before.

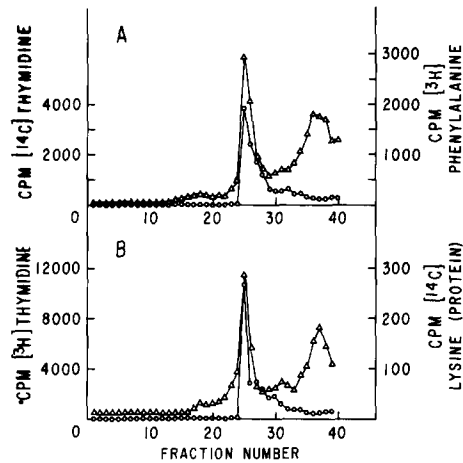


FIGURE 9 Centrifugation of homogenized nuclear suspensions labeled in their DNA (^3H - or ^{14}C thymidine) (O), and in their protein (Δ) (A, ^3H phenylalanine; B, ^{14}C lysine). The cells were labeled simultaneously with the two precursors for 12–20 h before nuclei were prepared as described. ^3H thymidine: 1 $\mu\text{Ci}/\text{ml}$, 20 Ci/mmol; ^{14}C thymidine: 0.1 $\mu\text{Ci}/\text{ml}$, 51.5 mCi/mmol; ^3H Phe: 5 $\mu\text{Ci}/\text{ml}$, 5.6 Ci/mmol; ^{14}C Lys: 0.5 $\mu\text{Ci}/\text{ml}$, 2.04 mCi/mmol; ^3H Leu: 3 $\mu\text{Ci}/\text{ml}$, 47 Ci/mmol. Conditions of centrifugation: 23 h, 25,000 rpm, 2°C.

We therefore tried to establish whether the material found in the peak fractions of gradients such as shown in Figs. 7, 8, and 9 was significantly contaminated with membranes or membrane fragments. Cells were labeled for 18–24 h with ^{14}C choline and nuclei were prepared as described in Materials and Methods. Only 5–8% of the total label incorporated in the whole cells remained associated with the nuclei, and more than 90% of these counts floated on top of the 30–60% gradient after 1–2 days of centrifugation, leaving 0.5% or less of the total ^{14}C choline associated with the chromatin band. When ^3H fucose was used as the label in an analogous experiment, again approximately 6% of the total label was found in the nuclear pellet, and one-third to one-half of these counts banded with the DNA.

DISCUSSION

We are presenting evidence that density gradients which can be formed by centrifugation of initially uniform solutions of Renografin, can cover the range of interest for the study of biological macromolecules. The limitation that the rate of formation of the equilibrium gradient is rather slow by comparison, for example, with cesium chloride

gradients, can be overcome by the use of preformed gradients. This initial condition can be chosen so that there will be only a small change upon centrifugation, and the gradient will then be maintained indefinitely during centrifugation. The corresponding solutions of Renografin are only slightly more viscous than aqueous solutions (a 30% solution has a relative viscosity of 1.12), but a

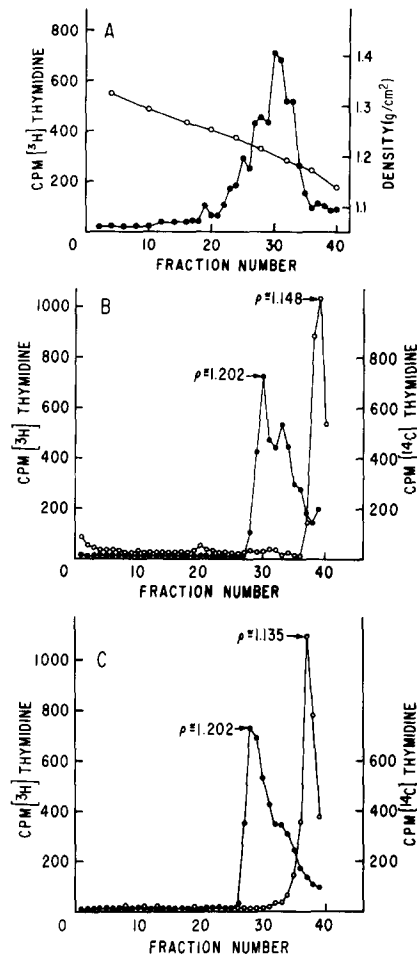


FIGURE 10 A Isopycnic banding of chromatin isolated by the procedure of Bonner et al. (1968). The chromatin was layered on top of a 30–60% linear gradient of Renografin and centrifuged for 40 h as described.

FIGURE 10 B and C Isopycnic banding of chromatin (\bullet) isolated by the procedure of Bhorjee and Pederson (1973). 41 h running time (B), and 91 h running time (C). Pure DNA (O) isolated from this chromatin was placed on identical gradients (30–60%) in separate centrifuge tubes and centrifuged for the same length of time. Conditions of centrifugation: 25,000 rpm, 2°C.

potential disadvantage of the use of such a solution is the extremely high absorbance in the ultraviolet wavelength region, necessitating a complete removal of the Renografin by dialysis or gel filtration before optical density measurements can be made to analyze a gradient.

The observed very low density of DNA in such a solution is rather striking. This may be due to the high degree of hydration of the DNA in such solutions which are less than 0.5 molar with respect to the major anion (3,5 diacetamido-2,3,6 triiodo benzoate). In part, the more abundant cation (*N*-methyl glucamine) may also be responsible for the observed low density, and it would be interesting to compare the observed densities with those observed in solutions containing only Na^+ as the cation.

We also note that T4 DNA is denser in such gradients than hamster DNA, although its GC content is lower, but we speculate that this may be explained by the presence of glucosylated bases in T4 DNA.

The expectation that under these conditions protein-DNA complexes might be stable is supported by our experiments. Material from homogenized or sonicated nuclei can be isolated reproducibly from the gradient at a density which is significantly greater than that of pure DNA, and similarly, chromatin isolated by conventional procedures (Bonner et al., 1968, Bhorjee and Pederson, 1973), is found to be denser than DNA in our conditions. This density shift could be caused by protein associated with the DNA, because the density of proteins ranges around 1.3 g/cm^3 and is not as dependent on the salt concentration as that of DNA. Another possibility is that the protein associated with the DNA prevents the extensive hydration observed with pure DNA, thus also causing an increase in density.

We do not know what is the precise nature of the association of the DNA with the RNA, but it seems clear that the RNA is not responsible for the density shift, since it can be completely digested with only a small effect on the density of the DNA-protein complex.

From our results with labeled choline or fucose, it appears that a small quantity of glyco- or lipoprotein remains associated with our material, but it is difficult to estimate which fraction of the total mass this represents.

A measurement of the ratio of absorbances at 260 nm and 280 nm of material isolated from the

gradient ($A_{260}/A_{280} = 1.2$) also indicates that a substantial amount of protein is present in the complex, but we have not yet scaled up our procedure to permit a more complete chemical analysis. A much more significant question at this time is whether the protein associated with the DNA reflects the *in vivo* situation.

When material from homogenized nuclei is run on a preformed gradient there is no indication of a rapid dissociation of the complex since the peak position is stable over several days of running time. On the other hand, isolation and manipulation of this material (dilution and some shearing) tends partly to destroy the complex. In this connection it should be noted that the homogenized nuclear suspension is not appreciably viscous as it is immediately placed on the centrifuge tube, but the peak fractions can almost always be detected before counting by their viscosity.

Experiments are in progress to characterize these nuclear proteins by gel electrophoresis with the aim of following the changes which occur during the cell cycle and specifically during mitosis, and our procedure will constitute a rapid, convenient, and reproducible first step in isolating chromatin-associated proteins. Compared with other methods our procedure may take some extra time on the centrifuge, but it may have the advantage of being usable with relatively small numbers of nuclei because manipulation of the chromatin is reduced to a minimum and losses due to the stickiness of the material are minimized.

Addendum

While this manuscript was in preparation a communication by Rickwood and Birnie (1973 *FEBS [Fed. Eur. Biochem. Soc.] Lett.* 33:221), came to our attention in which studies with a related compound, Metrizamide (2-[3-acetamido-5-*N*-methylacetamido-2,4,6 triiodobenzamido]-2-deoxy-D-glucose), were reported.

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