Differences of Supranucleosomal Organization in Different Kinds of Chromatin: Cell Type-specific Globular Subunits Containing Different Numbers of Nucleosomes

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ABSTRACT Fractions of homogeneously-sized supranucleosomal particles can be obtained in high yield and purity from various types of cells by brief micrococcal nuclease digestion (10 or 20 s) of condensed chromatin in 100 mM NaCl followed by sucrose gradient centrifugation and agarose gel electrophoresis. These chromatin particles, which contain only DNA and histones, differed according to cell type. Sea urchin spermatozoa (*Paracentrotus lividus*) gave rise to heavy particles (ca. 260 S) with a mean diameter (48 nm). These resembled the unit chromatin fibrils fixed in situ, which contain an average of 48 nucleosomes, as determined both by electron microscopy after unraveling in low salt buffer and gel electrophoresis. In contrast, higher order particles from chicken erythrocyte chromatin were smaller (105 S; 36-nm diam) and contained approximately 20 nucleosomes. The smallest type of supranucleosomal particle was obtained from chicken and rat liver (39 S; 32-nm diam; eight nucleosomes). Oligomeric chains of such granular particles could be recognized in regions of higher sucrose density, indicating that distinct supranucleosomal particles of globular shape are not an artifact of exposure to low salt concentrations but can be obtained at near-physiological ionic strength. The demonstration of different particle sizes in chromatin from different types of nuclei is contrary to the view that such granular particles are produced by artificial breakdown into "detached turns" from a uniform and general solenoid structure of approximately six nucleosomes per turn. Our observations indicate that the higher order packing of the nucleosomal chain can differ greatly in different types of nuclei and the supranucleosomal organization of chromatin differs between cell types and is related to the specific state of cell differentiation.

The question of how the nucleosomal chain, the basic structural element of organization of chromatin, is organized at the secondary level of chromatin packing remains unanswered. Two different models of supranucleosomal packing have been presented: (*a*) Finch and Klug (15) have proposed a continuous "solenoid" in which the nucleosomal chain is helically coiled, with about six nucleosomes per turn and a pitch of ~11 nm, thus forming a homogeneously thick fibril ~30 nm in diameter. Various observations have been interpreted to support this model (2, 4, 6, 7, 9, 10, 30, 31, 41, 53, 58). However, the majority of these experiments have been performed upon chromatin that has undergone destabilization through/by preparative "stress," such as lengthy dialysis and/or washes in buffers of drastically reduced ionic strength or containing chelating agents. According to this model, the solenoid should be identical in different kinds of condensed chromatin, irrespective of the cell type examined (31). (*b*) From electron microscopic observations of spread chromatin preparations and from studies of the products of limited digestion with micrococcal nuclease (16, 20, 24) other authors have proposed that the nucleosomal chain forms a discontinuous periodic structure appearing as a higher order chain of tightly adjoining globular aggregates ("superbeads," 42; "nucleomers," 25). This also results in the appearance of a higher order fibril, which displays an overall contour diameter of ~30 nm in many cell types (3, 8, 19, 21, 22, 25, 37, 39, 44, 52, 54, 66, 67, 68). This mode of supranucleosomal organization into higher order granules is similar to the organization

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 JULY 1984 272-286

that has been described for circular forms of chromatin, such as the SV40-"minichromosomes" (12, 23, 35, 59) and non-transcribed amplified rDNA chromatin of certain oocytes (46).

The solenoidal model has found more supporters in recent years (for references see 6, 11, 31). Experimental observations interpreted to support the discontinuous periodic arrangement in nucleomeric globules has been criticized for two reasons. Some authors have claimed that the structures reported as supranucleosomal units are not chromatin material but ribonucleoprotein complexes (6, 11, 36, 57, 61). However, in view of the high content of histones reported for the superbead particles (52), their occurrence in the nuclei of cells with extremely low RNA contents, such as avian erythrocytes and sea-urchin spermatozoa, and their reversible unfolding into extended chains of nucleosome-sized particles upon exposure to low ionic strength (e.g., 25, 52), this argument need not be discussed further. A more serious criticism of the biological significance of the superbead-like structures is based on the fact that most of the preparations used to demonstrate these granular structures also involve treatment of the chromatin at reduced salt concentrations. Therefore, Thomas et al. (4, 7) have proposed that such globular subunits represent "detached turns" of solenoid chromatin which are produced artificially during exposure to reduced ionic strength.

In the present study, we show that monodisperse fractions of globular supranucleosomal chromatin particles are also obtained in high yields after very short nuclease digestion in 100 mM NaCl, and that the nucleosomal contents of these globules differ in different cell types in a biologically meaningful manner. Our results indicate that the supranucleosomal organization of condensed chromatin is not identical in all types of nuclei, and that the packing of nucleosomes differ in different cell types and is probably related to cell differentiation.

MATERIALS AND METHODS

Animals: Sea urchins (*Paracentrotus lividus*) were collected in the Mediterranean Sea near Nice and kept in artifical sea water until use. Chickens were White Leghorn, 6–8-wk old or adult egg-laying hens. Rats were Sprague-Dawley (ca. 250 g body weight).

Isolation of Nuclei from Sea Urchin Sperm: Suspensions of freshly collected, living spermatozoa were carefully pipetted out of the opened gonades and washed twice in sea water (cf. 66). Use of frozen-stored sperm cells resulted in formation of gelatinous chromatin clumps and was avoided. For preparation of nuclei two alternative procedures were used. (*a*) Sperm cells were resuspended in a 50-fold volume of buffer A (100 mM NaCl, 0.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.4). While whirling on a Vortex mixer Nonidet-P40 (NP40) was added to give a final concentration of 0.5% and the cells were lysed for 5 min at 4°C with careful shaking of the tubes with ~30 s intervals. The homogenate was centrifuged for 10 min at 800 *g* and the sediment was resuspended in buffer A by vigorous action with a "medium-fitting" Dounce homogenizer. Examination by light microscopy revealed a clean nuclear fraction. Sperm tails and midpieces were not observed. To remove detergent the nuclei were washed twice in buffer A. This procedure did not always give sufficient yield of chromatin in the subsequent digestion step. Therefore, we also applied the following, more vigorous procedure: (*b*) Sperm cells were diluted with a 50-fold volume of 1 mM borate-buffer (pH 8.8). The solution was immediately vigorously shaken, kept on ice for 3 min, and centrifuged for 10 min at 800 *g*. Light microscopic control of the pellet did not reveal considerable contamination with sperm tails or midpieces. Finally the nuclei were washed twice in buffer A.

Isolation of Nuclei from Chicken Erythrocytes: Nuclei were prepared according to the method described by Weintraub (62) with the following modifications. Blood (6 ml) was obtained from hens by cardiac or vein puncture with a heparinized syringe. Blood was immediately diluted with a 10-fold volume of SSC-buffer (140 mM NaCl, 10 mM Tris-HCl, 15 mM Na-citrate, pH 7.4) and centrifuged for 10 min at 100 *g*. The supernatant was removed and the bottom layer containing the mature erythrocytes was washed twice in SSC. The final sediment was carefully resuspended by some strokes with a loosely-fitting Dounce homogenizer in 70 ml buffer A. While whirling on a Vortex mixer 0.35 ml of a 100% NP40 solution was added and the blood cells were lysed during 5 min (in 1 min-intervals the solution was carefully shaken by turns of the centrifuge tube). The homogenate was centrifuged for 10 min at 800 *g* and the sediment resuspended with the Dounce homogenizer in buffer A. The nuclei were washed twice in buffer A to remove the detergent, and the final sediment was controlled by light microscopy.

Isolation of Nuclei from Rat and Chicken Liver: Nuclei from liver tissue of both species were isolated as follows: Liver tissue was minced with scissors in cold (ca. 4°C) buffer D (0.44 M sucrose, 70 mM KCl, 10 mM Tris-HCl, pH 7.4, 2% purified gum arabic). The suspension was forced, using a press, through a fine metal sieve (mean pore size: 0.5–1 mm). Medium D was added to the homogenate, and the cells were disrupted with a Potter-Elvehjem glass-Teflon homogenizer (Braun Co., Melsungen, FRG). After centrifugation at 850 *g* (12 min), the pellet was resuspended with a loose-fitting Dounce-homogenizer, and the homogenate adjusted with 2.6 M sucrose (made in 100 mM NaCl, 10 mM Tris-HCl, pH 7.4) to 2.0 M and layered (15-ml portions) on top of a 20-ml cushion of 2.1 M sucrose (made in 100 mM NaCl, 10 mM Tris-HCl, pH 7.4). Centrifugation was performed in a SW 27 rotor (Beckman Instruments, Inc., Palo Alto, CA) for 90 min at 25,000 rpm at 4°C. The isolated nuclei recovered in the pellet were finally resuspended in buffer A by the use of a loose-fitting Dounce-homogenizer and washed twice in buffer A.

Preparation of Higher Order Chromatin Units: The isolation procedure for the higher order particles was identical for the four objects presented here. Nuclear sediments were resuspended in buffer C (100 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.25 mM phenylmethylsulfonyl fluoride, 10 mM Tris-HCl, pH 7.4) by several strokes in a loosely-fitting Dounce homogenizer and centrifuged for 10 min at 800 *g*. The sediments were resuspended in buffer C with a tightly-fitting glass-teflon homogenizer and adjusted to 50 A_{260} units per ml (read in 0.1 M NaOH). 0.5-ml samples were preincubated for 3 min at 37°C in a water bath, 100 U micrococcal nuclease dissolved in 40 μl buffer C were added, and enzyme digestion was for 10 s. In some experiments the digestion time was varied: 0, 10, 20, 40, 50, 60, 90, and 120 s. The reaction was terminated by the addition of 0.5 M EDTA or EGTA to a final concentration of 5 mM and the reaction vials kept on ice. In control experiments, chromatin was incubated in the same buffer without enzyme added. The 0.5-ml samples were loaded on top of preformed linear sucrose gradient (10–50% (wt/vol), 1 ml 70% cushion; sucrose solutions were made in buffer D, i.e., 100 mM NaCl, 1 mM EDTA, 0.25 mM phenylmethylsulfonyl fluoride, 10 mM Tris-HCl, pH 7.4). Centrifugation was performed in a SW 40 rotor (Beckman Instruments, Inc.) for 90 min at 40,000 rpm at 4°C. The tube content was fractionated (0.4-ml fractions) from the top and scanned with an ISCO UA-5 absorbance monitor (ISCO, Lincoln, NB) at 254 nm. For estimation of S-values in parallel gradients ribosomal subunits isolated from *Xenopus laevis* ovaries, phage φx 174 particles, and SV40 virions were centrifuged under identical conditions. Gradient fractions were immediately deep-frozen at −70°C, except those used for electron microscopy and agarose gel electrophoresis of chromatin.

In another set of experiments, fractions from the center peak region of sucrose gradients containing chicken erythrocyte chromatin were pooled, concentrated by vacuum dialysis in buffer D, and re-run in a sucrose gradient, using the same centrifugation conditions.

Similar experiments were done using combinations of chromatin particles from different sources. After concentration by vacuum dialysis to an appropriate volume equivalent amounts of chromatin were mixed and re-centrifuged in the following combinations: sea urchin sperm × chicken erythrocyte, chicken erythrocyte × chicken liver, and chicken erythrocyte × rat liver.

Additionally chicken erythrocyte chromatin was digested with micrococcal nuclease as described above, dialyzed overnight against low salt buffer (1 mM EDTA, 10 mM Tris-HCl, pH 7.4) and examined by sucrose gradient centrifugation (in 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) as described above.

As a control, purified DNA from sea urchin, chicken and rat was treated (same A_{260} units) with micrococcal nuclease.

Chemical Determinations: Nucleic acids and proteins were determined as described (64).

Agarose Gel Electrophoresis of Chromatin: Chromatin particles were applied to 3-mm thick 0.3% agarose gels. The buffer for loading and electrophoresis was 40 mM Tris-HCl, 20 mM Na-acetate, 2 mM EDTA (pH 7.9). The gels were run at 5 V/cm and DNA was stained with ethidium bromide.

DNA Analyses: Gradient fractions or chromatin portions were incubated in the presence of pronase (0.25 mg/ml) and Sarkosyl (1%) for 5 h at 37°C. DNA was extracted with buffer (10 mM Tris-HCl, pH 7.4)-saturated

phenol, followed by extraction with a mixture (1:1) of phenol-chloroform/isoamyl alcohol (24:1) and precipitated with ethanol. DNA was subjected to electrophoresis in horizontal 1% (wt/vol) agarose gels and run in 40 mM Tris-HCl, 40 mM Na-acetate, 2 mM EDTA (pH 8.3). The gels were calibrated with restriction fragments of λ-DNA (*Hind III*) and fragments of ϕx 174 RF-DNA (*Hae III*), and slots were scanned by the use of a Joyce-Loebl Chromoscan 3 (Joyce-Loebl Instruments, Duesseldorf, FRG).

Protein Analyses: SDS PAGE was carried out essentially according to Thomas and Kornberg (56). Chromatin samples were precipitated overnight at 4°C by the addition of ice-cold trichloroacetic acid (TCA) to a final concentration of 20%. After centrifugation the material was washed first with 90% acetone containing 0.1 N HCl, then with acetone. Dried samples were dissolved in sample buffer, heated, and applied to the gel. Gels were stained either with Coomassie Blue or with silver nitrate using the method of Switzer et al. (55). Proteins were fixed in the gel with 10% TCA, followed by incubation in a mixture of methanol, acetic acid, and water (5 : 1.2 : 3.8; vol/vol/vol). The gel was washed several times in distilled water and postfixed for 15 min in 1% glutaraldehyde buffered with 50 mM sodium borate (pH 9.4). After extensive rinsing in water, the gel was incubated in a solution containing 0.8% silver nitrate, 0.5% ammonia, and 20 mM NaOH, rinsed in water, and processed as described by Switzer et al. (55). Densitometer tracing of Coomassie Blue-stained polypeptide bands was performed with the Joyce-Loebl instrument as described above.

Preparation of Chromatin for Electron Microscopy: Chromatin spread preparations were performed according to the procedures described earlier (16, 33, 47) with the following modifications. Chromatin samples from sucrose gradient fractions were immediately fixed with glutaraldehyde (final concentration 0.2%) for at least 60 min at 4°C. In some cases the chromatin samples were diluted in a buffer of a chromatin-stabilizing ionic strength (0.1 M NaCl, 10 mM Tris-HCl, pH 7.4 or 0.1 M NaCl, 10 mM TES (TES-(*N*-tris[hydroxymethyl]methyl-2-aminoethane sulfonic acid))-HCl, pH 7.4) and fixed in suspension (same final concentration). Chromatin was then centrifuged on electron microscopic grids as described by Scheer et al. (47), except a modified sucrose cushion in the centrifuge chamber. In all spreading experiments 0.1 M sucrose solutions contained only 0.1 M NaCl or, in addition, 0.2% glutaraldehyde or 1% formaldehyde, buffered with 10 mM TES-HCl (pH 7.4) or 10 mM Tris-HCl (pH 7.4). Staining of the specimens was as described earlier (47). For contrast enhancement most of the specimens were rotary-shadowed with platinum/palladium (80:20) at an angle of 8°. Alternatively, spread particles were metal shadow-cast with platinum/palladium (80:20) under an angle of 8°. For negative staining the specimens centrifuged on electron microscopic grids were rinsed for 1 min in double-distilled water and stained with aqueous 2% uranyl-acetate, which contained one drop dimethyl sulfoxide per ml staining solution. Positive staining with phosphotungstic solution was as described (16, 18). For unraveling into nucleosomal chains chromatin was solubilized in low salt buffer (45) and processed as described (47). For electron microscopy of ultrathin sections, tissue samples, pelleted cells, isolated nuclei, and chromatin fractions were fixed in 2.5% glutaraldehyde buffered with 0.05 M sodium cacodylate (pH 7.0; for fixation in suspension 5% glutaraldehyde was used). Osmication, dehydration, embedding, and sectioning were as described (17). Relatively thick sections (50–80 nm) were used for measurements of fibril widths.

Micrographs were taken with a Zeiss EM-10 electron microscope at 40, 60, and 80 kV, respectively. The magnification indicator was routinely controlled by the use of a grating replica. Measurements of the diameter of chromatin structures, counting of nucleosomes, length measurements, and calculations were performed as described (46).

RESULTS

In the course of our electron-microscopic and biochemical studies on the organization of condensed chromatin, we encountered differences of chromatin fibril dimensions in different cell types. For the sake of clarity, we have selected three extreme cell types: sea-urchin spermatozoa, chicken erythrocytes, and hepatocytes of chick and rat, i.e., cell types that have been widely used in chromatin research.

Electron Microscopy of Fixed Cells and Isolated Nuclei

Examining the dimensions of chromatin structures in sections of nuclei of fixed intact cells and isolated nuclei, we noted that the diameters of the uniform chromatin fibrils of sea-urchin spermatozoa of *Paracentrotus lividus* were larger (37 nm) than those present in chicken erythrocytes (28 nm; Fig. 1) and the peripheral condensed chromatin of chicken and rat hepatocytes (26 nm; data not shown; Table I). The greater diameters of sea-urchin sperm chromatin fibrils can also be seen in other sea urchin species (e.g., 29). Our value of 28 nm for erythrocytes and other cells agrees with the "unit thread" diameter estimated by Davies and colleagues (13, 14, 60). The chromatin structures appeared uniformly stained with lead citrate as well as with uranyl acetate and never revealed an electron-transparent central core (Fig. 1, *a–e*). Center-to-center spacings of fibrils of sea-urchin sperm chromatin were also much larger than those of chicken erythrocyte and liver chromatin (Table I). When the fibril diameters were corrected for shrinkage due to fixation, dehydration, and embedding (cf. 28), it became apparent that the native fibrils of sea-urchin sperm chromatin are larger (46 nm) than expected for a solenoid containing six nucleosomes per turn. In grazing sections, the chromatin fibrils often exhibited somewhat knobby outer contours (see below and Fig. 12*d*; cf. 65).

Supranucleosomal Particles Obtained after Limited Nuclease Treatment

We have developed procedures that produce optimal yield of higher-order subunits from digested chromatin. When chromatin samples of nuclei isolated from different cell types were digested for very short periods of time (10 or 20 s) and maintained in 100 mM NaCl, chromatin was obtained which appeared as a monodisperse population of particles on sucrose gradients (Fig. 2). In evaluating these results, it should be kept in mind that in our experiments the total digest preparation was applied to the sucrose gradients without prior separation of " solubilized chromatin," as in most other reports (e.g., 4, 7, 43). Recoveries of chromatin in the main peak particle fraction, as estimated from determinations of DNA or total absorbance at 254 nm, were 40%–50% for sea-urchin sperm (12 different experiments) and 70%–85% for other cell types examined. Therefore, we conclude that these particle fractions represented most of the condensed chromatin of these cells and did not originate from a selected special type of chromatin fibril. Comparison of the sedimentation properties of these particles revealed marked differences (Fig. 2, Table I). Chromatin particles from sea-urchin sperm sedimented much faster (estimated value of 260 S) than those from chicken erythrocytes (105 S), and this difference was also seen on recentrifugation of the pooled material from the peak fractions and on co-centrifugation of mixtures of these particles (Fig. 2, *A–D*). Our value of 105 S for chicken erythrocyte chromatin particles is similar to that reported by Puigdomenech and Ruiz-Carillo (40), albeit after much longer digestion times (1 h). On the other hand, particles dissected from liver chromatin of chicken and rats were significantly smaller (39 S) than the avian erythrocyte chromatin particles, and this difference was also demonstrable by centrifugation of particle mixtures (Fig. 2, *E–H*).

Using the same digestion conditions in 100 mM NaCl, the specific S values of the supranucleosomal particles did not significantly decrease after further treatment with micrococcal nuclease (Fig. 3, *A–F*; see also 40). The only result of this prolonged digestion was an increase in the proportion of nucleosomal particles, at the expense of the higher order particles, and a slight increase in the modal width of the

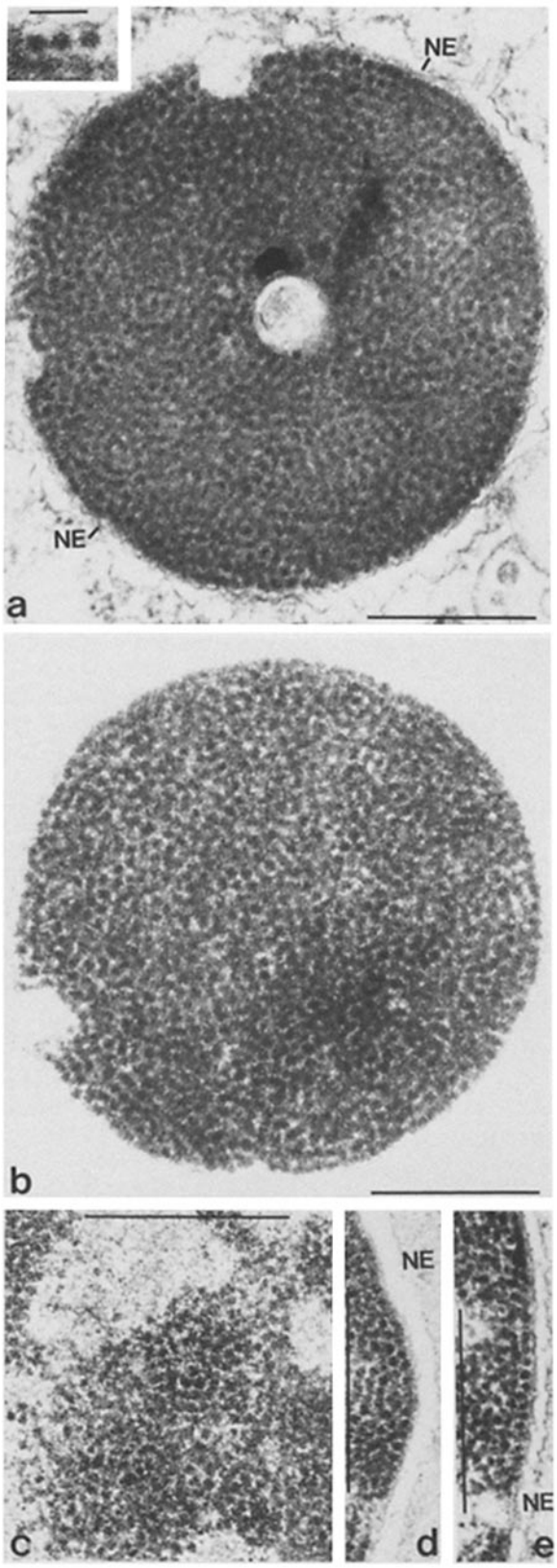


FIGURE 1 Electron microscopy of highly condensed chromatin in situ as revealed in cross sections through spermatozoa of the sea urchin, *Paracentrotus lividus*, at the level of the centriolar fossa (*a*), through isolated nuclei from these cells (*b*), and through nuclei of avian erythrocytes (*c–e*). Note the uniform organization of sea urchin sperm chromatin in closely packed, regularly-spaced and -sized, 37-nm large globular subunits that are surrounded by unstained "halos," which probably result from shrinkage during the preparation (*a* and *b*). The total diameter of the unit chromatin thread corrected for shrinkage is 46 nm. A similar granulofibrillar organization of the condensed chromatin can also be observed in ultrathin sections in various planes of sectioning through chromatin of inner regions (*c*) and the periphery (*d* and *e*) of chicken erythrocytes but here the chromatin consists of 28-nm thick structures (36 nm after correction for shrinkage). The outermost layer of regularly arranged chromatin fibrils is tightly attached to the inner membrane of the nuclear envelope (*NE*). Bars, 0.5 μm (*a–e*); 0.1 μm (*inset* in *a*). × 46,000 (*a* and *b*); × 83,000 (*inset* in *a*); × 56,000 (*c–e*).

particle peak. Likewise, a second digestion for 10 s of particles collected from the peak fractions did not result in a significant drop of their S values (not shown).

We have also controlled the possible contribution from endogenous nuclease activities to the enzymatic chromatin dissection by parallel incubations without the addition of micrococcal nuclease. Fig. 3*G* shows that, under these conditions, no significant amounts of defined chicken erythrocyte chromatin particles appear during 20–120 s. A somewhat higher proportion of digested chromatin material was found in the liver nuclei from both species (Fig. 3*H* presents the example of chicken liver), but this did not result in the appearance of a pronounced particle peak at 39 S. Therefore, we conclude that the formation of the monodisperse particles from the specific types of chromatin is due to the action of the micrococcal nuclease added.

Electron Microscopy of Supranucleosomal Subunit Particles

To show the purity and homogeneity of the chromatin particles obtained, we present large representative survey pictures rather than small selected cut-outs as they are frequently offered in the literature.

Fractions obtained after sucrose gradient centrifugation of chromatin briefly digested with micrococcal nuclease in 100 mM NaCl (Fig. 2) were prepared for electron microscopy using different methods. Particle size differences between the different types of chromatin particles were obvious. In spread preparations the particles of the 260 S peak fractions from sea urchin spermatozoa appeared as a rather uniform population of globular shape (Fig. 4, *a–g*), and the appearance was similar with (Fig. 4, *b* and *c*) and without (Fig. 4*a*) glutaraldehyde fixation, after rotary metal-shadowing (Fig. 4, *a* and *b*), after postitive (not shown), and after negative (Fig. 4, *c–g*) staining. The mean diameter measured in metal-shadowed preparations was 55 nm, i.e., 48 nm after correction for metal deposition. A diameter of 48 nm was also determined for positively or negatively stained particles. At higher magnification the negatively stained particles revealed a finely granular composition, suggestive of a moruloid organization (Fig. 4, *d–g*). In some places, these granular subcomponents were identified as distinct 10–15-nm particles, interpreted to be nucleosomes (Fig. 4, *e–g*). Only occasionally, elongated cylindrical particles with an indication of helical organization were detected, and these were usually characterized by a looser packing of subcomponents (bracket in Fig. 4*f*). Fractions taken from the heavy shoulder of the 260 S peak contained dimers and oligomeric chains of serially arranged 48-nm particles (Fig. 4 h; see also below). After exposure of the 48 nm chromatin particles to low salt buffer they unraveled and extended into nucleofilaments, sometimes with helically-appearing intermediate structures (Fig. 4, *i–j*). An average num-

TABLE I

Dimensions of Higher Order Chromatin Particles

Cell Type	Diameter in situ	Diameter*	Center-to-center spacing*	Diameter of isolated particles‡	Diameter of isolated particles§	Mean number of nucleosomes per particle	Mean DNA content per particle	Approximate S-value of isolated particles
	nm	*nm*	*nm*	*nm*	*nm*		*10^3 bp*	
Sea urchin sperm	37 ± 4	46	49 ± 5	56 ± 5	48 ± 5	48	11.5	260
Chicken erythrocyte	28 ± 4	36	36 ± 5‖	41 ± 4	35 ± 4	20	4.3	105
Chicken liver	26 ± 5	36	36 ± 2	36 ± 2	32 ± 2	8	1.5	39
Rat liver	26 ± 3	35	36 ± 3	36 ± 2	32 ± 2	8	1.6	39

* Corrected for dehydration and shrinkage (cf. 27, 28).

‡ From spread preparations.

§ Corrected for metal deposition during shadow cast; these values were not significantly different from those determined by positive staining with phosphotungstic acid alone, i.e., without shadowing.

‖ A value for 35 nm has been reported previously (65).

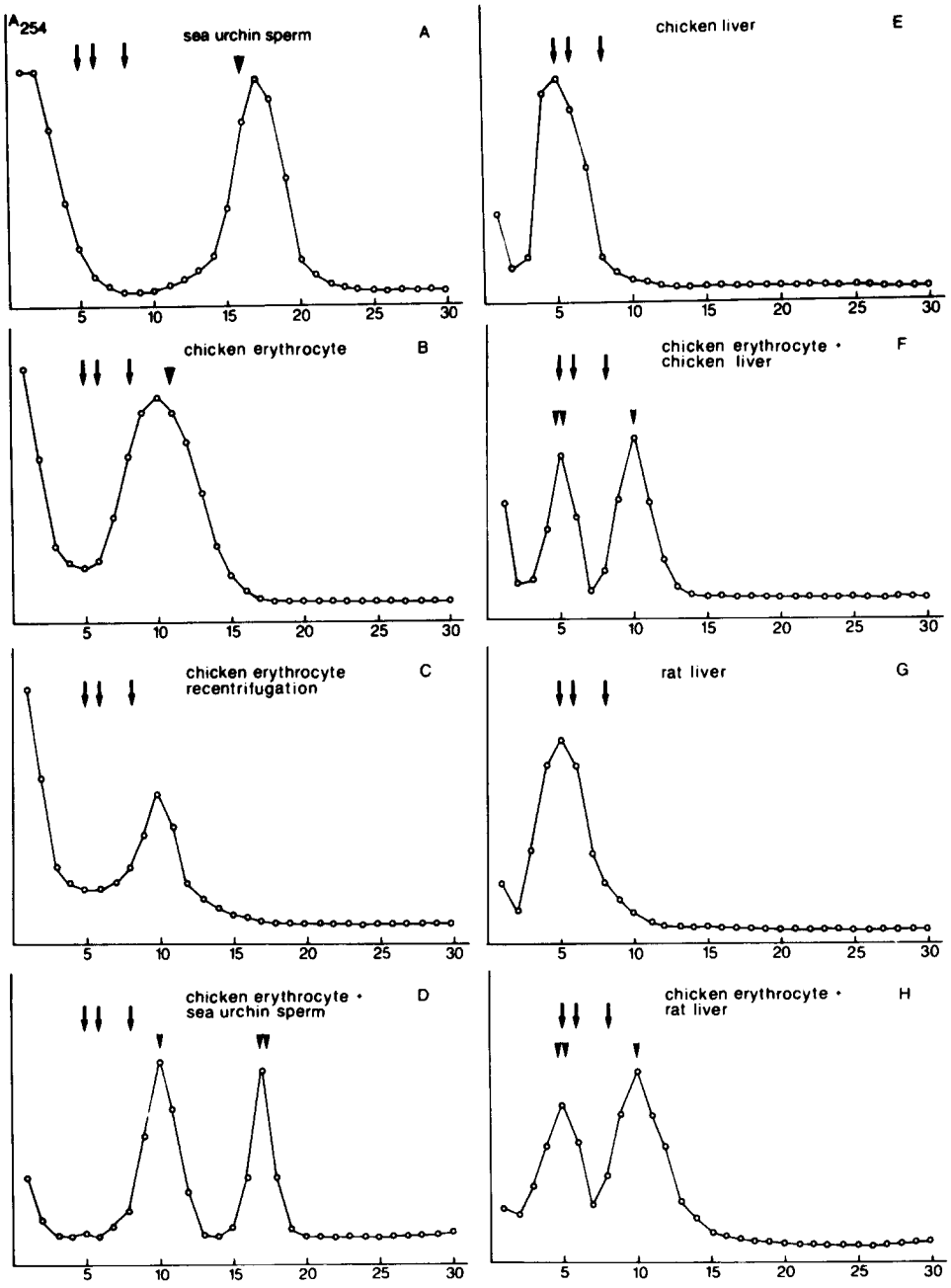


FIGURE 2 Sedimentation analysis of supranucleosomal chromatin particles in sucrose gradients. The total chromatin digested for 10 s in 100 mM NaCl was applied to sucrose gradients containing 100 mM NaCl. The estimated sedimentation coefficients of the peaks obtained with the different types of chromatin were determined to be ~260 S (sea urchin sperm, *A*), 105 S (chicken erythrocyte, *B*), and 39 S (chicken liver, *E*, and rat liver, *G*). The positions of the ribosomal sedimentation markers, i.e., ribosomes and ribosomal subunits from *Xenopus laevis*, are denoted by arrows (39 ± 2 S; 59 ± 2 S; 80 ± 2 S; cf. reference 38; sedimentation is from left to right). Triangle in *A* denotes the position of SV40 virions used for reference (240 S; cf. reference 5); triangle in *B* denotes the position of ϕx174 particles (114 S; cf. reference 63). For demonstration of homogeneity the material contained in the peak fractions 9–11 of the chicken erythrocyte chromatin (*B*) was recentrifuged under identical conditions, again revealing an average sedimentation coefficient of 105 S (*C*). The differences of sedimentation properties were best shown when peak fractions from different sources of chromatin were mixed and recentrifuged. (*D*: fractions 15–19 of *A* mixed with fractions 8–12 from *B*; *F*: fractions 8–12 from *B* mixed with fractions 4–7 from *E*; *H*: fractions 8–12 from *B* mixed with fractions 4–7 from *G*). The single arrowheads in *D*, *F*, and *H* denote the position of chicken erythrocyte chromatin; double arrowheads denote in *D*, the position of chromatin of sea urchin sperm, in *F*, chicken liver, and in *H*, rat liver, respectively.

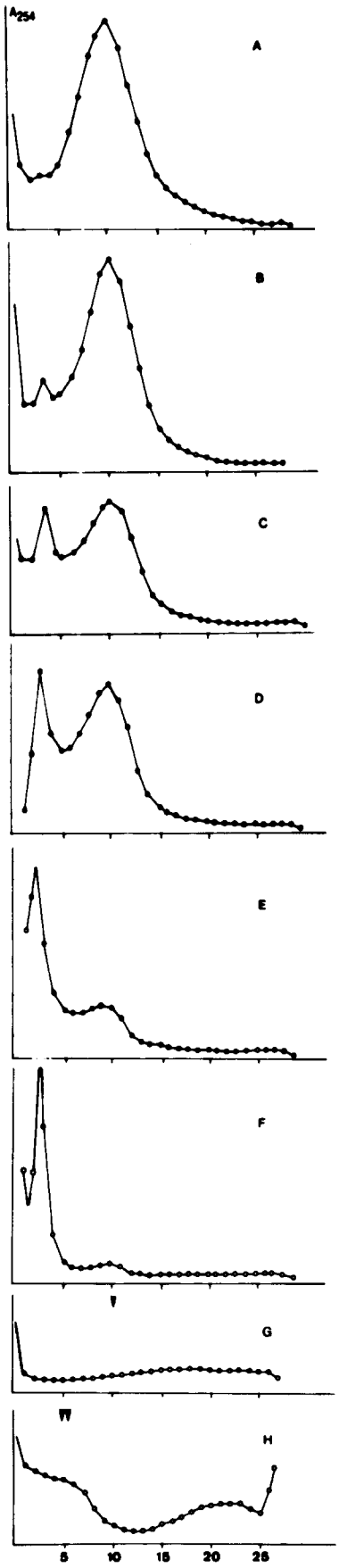


FIGURE 3 Sedimentation analyses of chromatin particles obtained by prolonged digestion with micrococcal nuclease (*A*, 20 s; *B*, 40 s; *C*, 50 s; *D*, 60 s; *E*, 90 s; *F*, 120 s) and of products observed in control experiments in which chromatin from chicken erythrocytes (*G*) and liver (*H*) has been incubated in buffer *C* without addition of micrococcal nuclease. Note that the position of the peak containing the higher order chromatin particles (fraction no. 10) does not shift with prolonged DNA digestion, whereas the height of the specific peak, i.e., the total higher order particle material, gradually decreases. Correspondingly, an increasing proportion of the chromatin appears in the characteristic nucleosomal peak (fraction 3). The control experiments without nuclease added show the absence of peak fractions containing higher order chromatin particles of avian erythrocytes (*G*, fraction 10; position of higher order chromatin particle peak is denoted by arrowhead) and liver (*H*; fraction 5; position of particles denoted by pair of arrowheads). In the experiments shown in *G* and *H* the bulk of the chromatin was recovered in the specific pellets (not included).

ber of 48 nucleosomes was counted per particle unit, and internucleosomal "spacer" DNA was sometimes recognized (Fig. 4*k*).

The 105 S chromatin particles from chicken erythrocytes were also found to be a very pure and homogeneous fraction that, in metal-shadowed (Fig. 5*a*), in unshadowed, positively (Fig. 5*b*), and negatively (Fig. 6*a*) stained preparations, appeared as granules of 36-nm diam (Table I). The overall appearance of negatively stained particles was moruloid, and individual nucleosomes were often discerned at higher magnification (various aspects are shown in Fig. 6, *b–e*). When exposed to low salt buffer the particles unfolded into extended nucleosomal chains containing an average of 20 nucleosomes (Fig. 6, *f* and *g*). Fractions 13–16 also contained short chains of two to five of these particles in close packing (not shown).

The much slower sedimenting particles of the 39 S peak fractions from liver tissue of rat (Fig. 7, *a* and *b*) and chicken (Fig. 7*d*) were also homogeneous. However, in both species the mean sizes of the particles were slightly less (32 nm), and unraveling in low salt buffer revealed only short nucleofilament units containing an average of eight nucleosomes (Fig. 7, *c* and *e*), identical with earlier determinations of Strätling et al. (52) and Kiryanov et al. (25).

When the chromatin particles from all three cell types were metal shadow-cast under defined angles, the shadow lengths were almost identical in a given fraction, indicating that all particles were isodiametric and not cylindrical (Fig. 8 presents the example of the erythrocyte chromatin particle fraction 10).

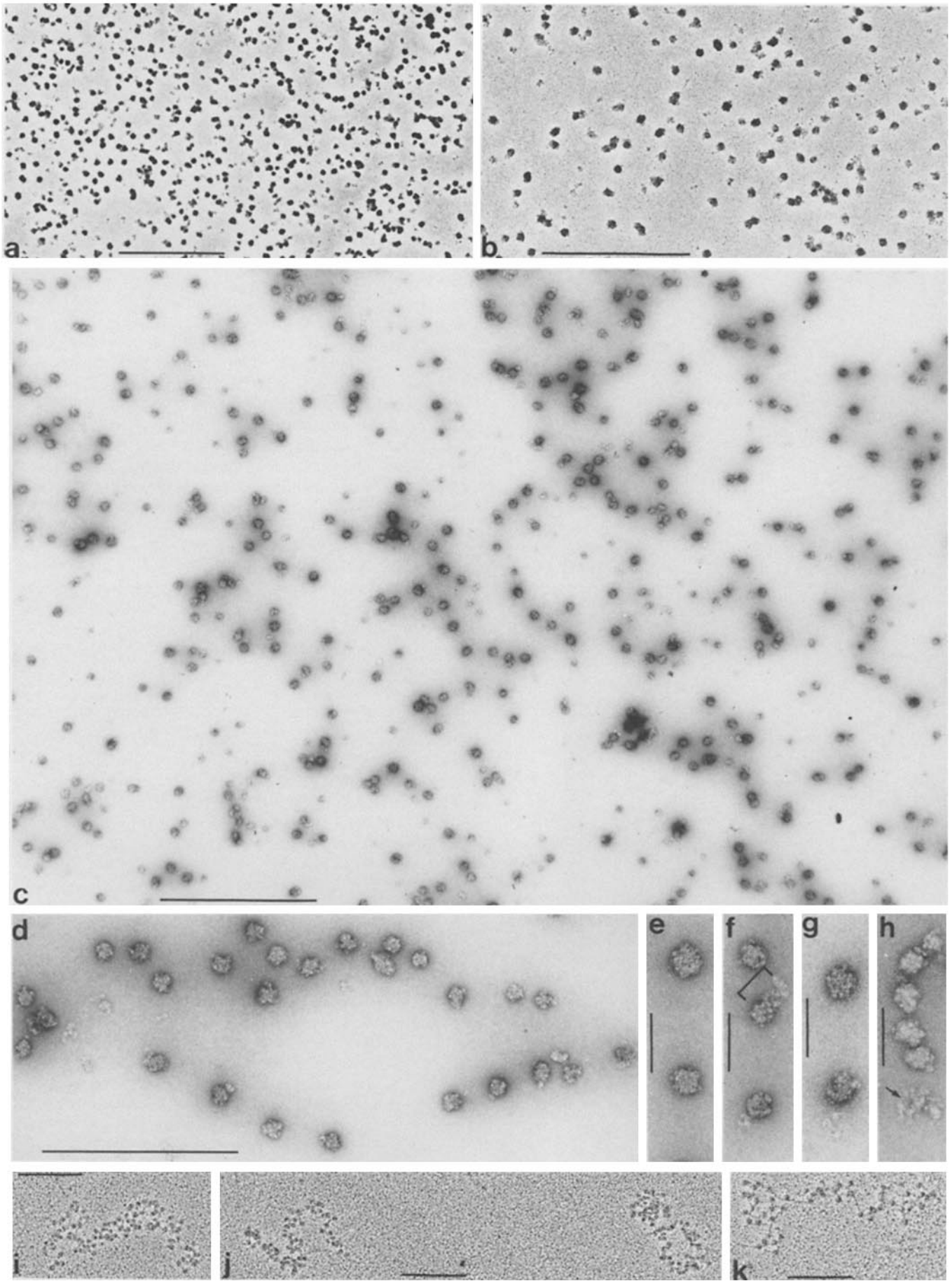
The quantitative evaluations of such preparations are summarized in the histograms of Fig. 9, *A–D* which show the different nucleosomal contents of the supranucleosomal particles from the different types of chromatin examined. The larger chromatin particles from chicken erythrocytes and sea urchin sperm consistently displayed somewhat wider distribution of sizes.

Particle Separations in Agarose Gels

Supranucleosomal particles obtained after very short micrococcal nuclease digestion could also be separated by electrophoresis in agarose. For example, Fig. 10*a* presents the 105 S particles from chicken chromatin which migrated as a well-defined band. The particles from the different cell types examined showed different electrophoretic mobilities: those from rat and chicken liver were the fastest; those from sea urchin sperm were the slowest.

Determinations of Lengths of DNA in Higher Order Subunit Particles

When DNA extracted from the various particle fractions separated by sucrose gradient centrifugation or agarose gel electrophoresis was analyzed by gel electrophoresis, multiples of nucleosomal equivalents were found in upper fractions whereas a characteristic maximum value of particle DNA was attained in the specific peak fractions (Fig. 10, *b–d*). Comparison with molecular weight reference samples showed that the 105 S chromatin particles of the peak fractions obtained by digestion of chicken erythrocyte chromatin contained 4.3 kb of DNA which, on the basis of a value of 212 bp per nucleosome (34), represents 20 nucleosomal equivalents (Fig. 10*b*). The stability of this particle DNA was demonstrated in experiments in which the particles obtained after brief digestion were dialyzed overnight against 1 mM Tris-HCl (pH 7.2) containing 1 mM EDTA and then separated by sucrose gradient centrifugation in the same low salt buffer. The peak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fraction, which sedimented at a lower S-value (ca. 60 S) as expected (25), still contained 4.3 kb DNA (Fig. 10*c*). Correspondingly, the larger (260 S) chromatin particles obtained in the peak fraction from sea urchin spermatozoa contained 11.5 kb of DNA (Fig. 10*d*) which, assuming a nucleosomal content of 242 bp (31, 50), is equivalent to 48 nucleosomes (Table I). The 39 S particles obtained from rat and chick liver contained 1.5 and 1.6 kb DNA (not shown), in agreement with values reported by Strätling et al. (52) and Kiryanov et al. (25). In all species examined digestion of purified DNA (see Materials and Methods) resulted in much smaller and less-defined fragments (data not shown).

Analysis of Proteins of Higher Order Particles

SDS PAGE of the peak fractions obtained by sucrose gradient centrifugation or by agarose gel electrophoresis demonstrated the nucleohistone nature of these particles (Fig. 11). In addition to the nucleosomal core histones, the proteins detected included the H1 representatives of liver chromatin, H1 and H5 of chicken erythrocytes, and the sea urchin sperm-specific histones (Fig. 11) of the H1 and H2 families (cf. 49).

Nonhistone proteins were present in minor amounts, usually <5% of the total Coomassie Blue-stained material recovered on gel electrophoresis. In preparations from liver tissue and chick erythrocytes, small amounts of nuclear lamins, identified by gel immunoblotting technique, were among the more frequent nonhistone proteins. Using densitometry of stained gels we compared the proportions of the individual histones in the isolated nuclei and in the chromatin particle fractions. The results showed similar proportions of histones of the H1 and H5 families to the nucleosomal core histones in both whole nuclei and higher order particles (Fig. 11*a*, lanes *1* and *2* shows such a comparison for chicken erythrocytes, and Fig. 11*b* presents the corresponding densitometer tracing of lane *2*), demonstrating the preservation of H1 and H5 histones during the preparation.

Oligomers of Higher Order Particles

When fractions were collected from portions of the sucrose gradients heavier than the main peak (fraction nos. 20–30 for sea urchin sperm; fraction nos. 13–20 for chicken erythrocytes; fraction nos. 8–15 for rat and chicken liver) and examined by electron microscopy and gel electrophoresis of DNA, a preference for oligomeric units was noticed. In some experiments these fractions were centrifuged again, using the same gradient system, to enrich for distinct oligomeric particle subclasses. For rat and chicken liver, using slightly different conditions of preparation involving a buffer of lower ionic strength (25, 52), we obtained similar values to those previously reported from this and other laboratories, indicating the existence of multiples of eight nucleosomes and approximately 190 bp (data not shown). Chains of two, three or four particles of ca. 35 nm in close apposition were found in "heavy particle fractions" from gradient separations of chicken erythrocyte chromatin (Fig. 12, *a–c*). Frequently the isolated dimers and oligomers revealed the DNA linker stretch interconnecting the globular subunits (arrowheads in Fig. 12*b*). The larger particles appeared as knobby fibrils of a morphology similar to that of the higher order chromatin fibril fixed in situ (compare Fig. 12, *c* and *d*). On exposure to lower salt the particle chains unraveled into long nucleofilaments containing average numbers of nucleosomes expected for dimers and trimers (fractions 14–16 of chicken erythrocyte chromatin, for example, showed chains of an average of 38 nulceosomes; values from fractions containing trimers and longer chains were less sharply distributed). Analysis of the DNA contained in such fractions revealed peaks corresponding to 4.3, 6.5, and 9.4 kb, equivalent to average values of 20, 31, and 44 nucleosomal DNA units (Fig. 12*e*). While the peaks of 4.3 and 9.4 kb could be related to monomers and dimers of the 34-nm chromatin particles, the preferential cleavage at 6.5 kb is so far unexplained. It should be noted, however, that the integrity of the intraglobular DNA is not necessary for maintenance of size and shape of these particles which appear to be organized by histone-histone interactions in the organization of "superbead"-like subunits (43).

Sizes of DNA molecules extracted from oligomeric chains of higher order chromatin particles from sea urchin spermatozoa (e.g., Fig. 4*h*) were very high (ca. 24 kb for dimers) and more difficult to determine.

DISCUSSION

We have taken great care to work out a nuclease digestion protocol which allows the dissection of condensed chromatin under conditions presumed to minimize artifacts, i.e., at near physiological ionic strength and for very brief periods of time. The procedure that we describe here is reproducible and effective, resulting in high yields of a pure and rather homogeneous fraction of globular supranucleosomal particles. The particles obtained are of discrete sizes and do not decrease on further digestion at this ionic strength, indicating that they are products of preferential cleavage and are not due to nonspecific artifacts at reduced ionic strength, such as disruption of solenoid fibrils into "detached turns" of six nulceosomes (4, 7, 26). Although a knobby appearance is sometimes suggested in chromatin fibrils encountered in sections through nuclei, we can presently not decide whether the formation of discretely-sized higher order particles reflects a principle of

FIGURE 4 Electron microscopy of spread preparations of supranucleosomal subunits isolated from sea urchin sperm chromatin. Pooled peak fractions (nos. 16–18) from sucrose gradient were used without (*a*) and with (*b–h*) glutaraldehyde fixation or after dilution with low ionic strength buffer (*i–k*) to induce unfolding of the nucleosomal chain. Preparations were stained with uranyl acetate (*c–h*) or shadow-cast with platin/palladium (*a*, *b*, and *i–k*). Note the size homogeneity of particles. Negative staining reveals the moruloid organization of the particle surface (*d–h*) and internal granular substructures, apparently nucleosomes (*e–g*). Occasionally, cylindrical particles with a suggestion of helical organization are seen (bracket in *f*). Oligomeric chains of tightly apposed supranucleosomal subunits are observed in fractions taken from the "heavy shoulder" of the peak (*h* shows an example in fraction no. 22 of Fig. 2*A*; arrow denotes a partially unraveled particle). On exposure to low ionic strength the higher order globules unfold into extended chains of nucleosomes (mean content 48 nucleosomes; *i* and *j*) which sometimes reveal their connection by spacer DNA (*k*). Bars, 1 μm; (*a–c*); 0.5 μm; (*d*); 0.1 μm (*e–h*); and 0.2 μm (*i–k*). × 19,500 (*a*); × 27,500 (*b*); × 28,500 (*c*); × 72,500 (*d*); × 110,000 (*e*, *f*, and *h*); × 112,000 (*g*); × 60,000 (*i–k*).

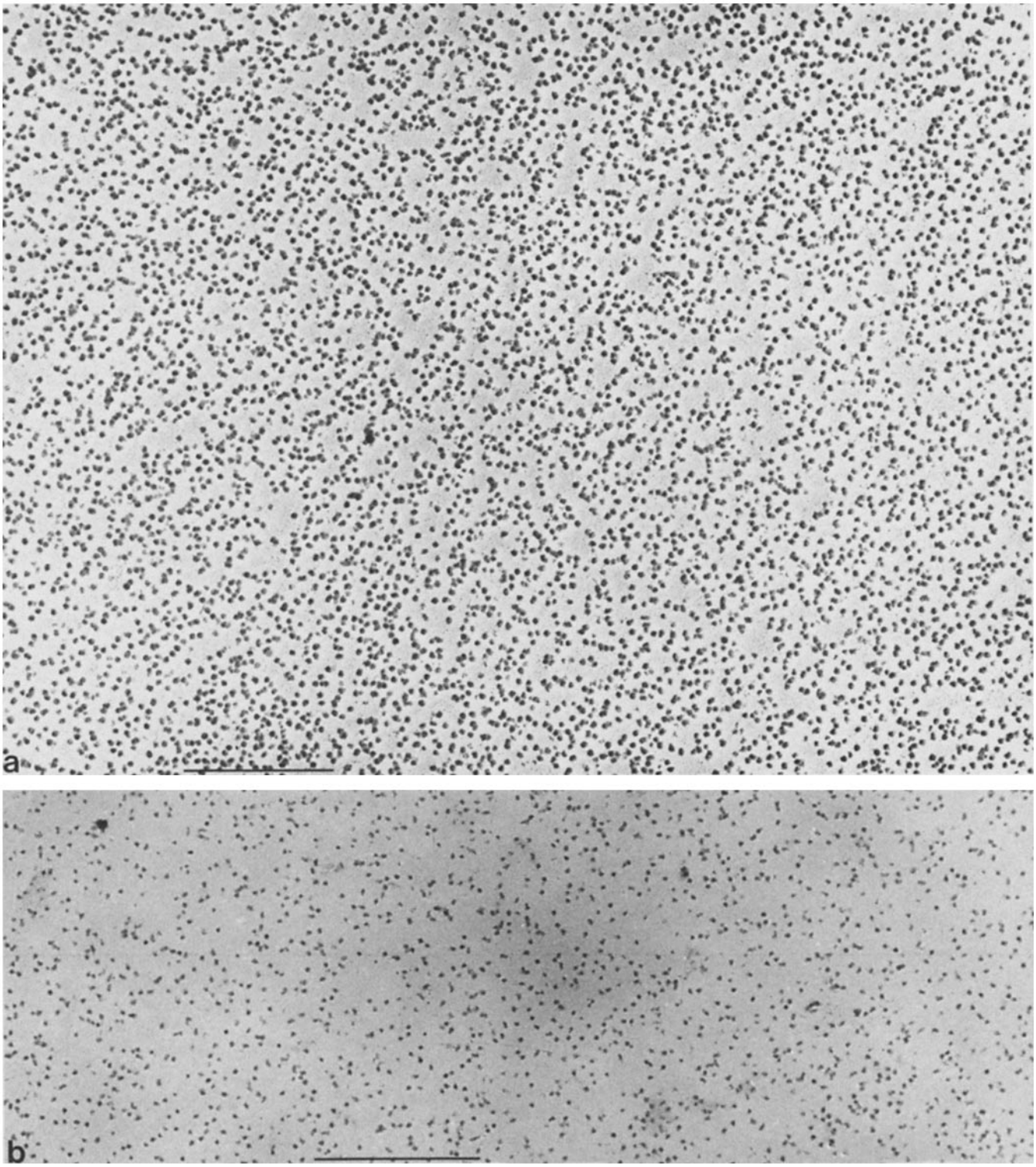

FIGURE 5 Electron microscopy of sucrose gradient centrifugation fractions of higher order particles from chicken erythrocyte chromatin. Material obtained from the main peak fraction (no. 10) of a sucrose gradient was fixed with glutaraldehyde and spread for electron microscopy. Note the purity and the homogeneous sizes of the globular particles. The same preparation is shown after positive staining, followed by metal-shadowing (*a*), and after positive staining with phosphotungstic acid alone (*b*), revealing no significant structural difference in the appearance of the chromatin particles between the two methods. Note increased particle diameters in *a* due to metal deposition. Bars, 1 μm (*a* and *b*). (*a*) × 25,000; (*b*) × 28,000.

internal organization rather than a distinct subunit of the chromatin fibril in the living cell. The finding that supranucleosomal particles of different sizes and nucleosomal contents are obtained from different cell types provides an additional argument that such particles do not originate from a general artifact of chromatin preparation but are related to some kind of differences between subunits of chromatin packing in these cells. Moreover, the pronounced size differences

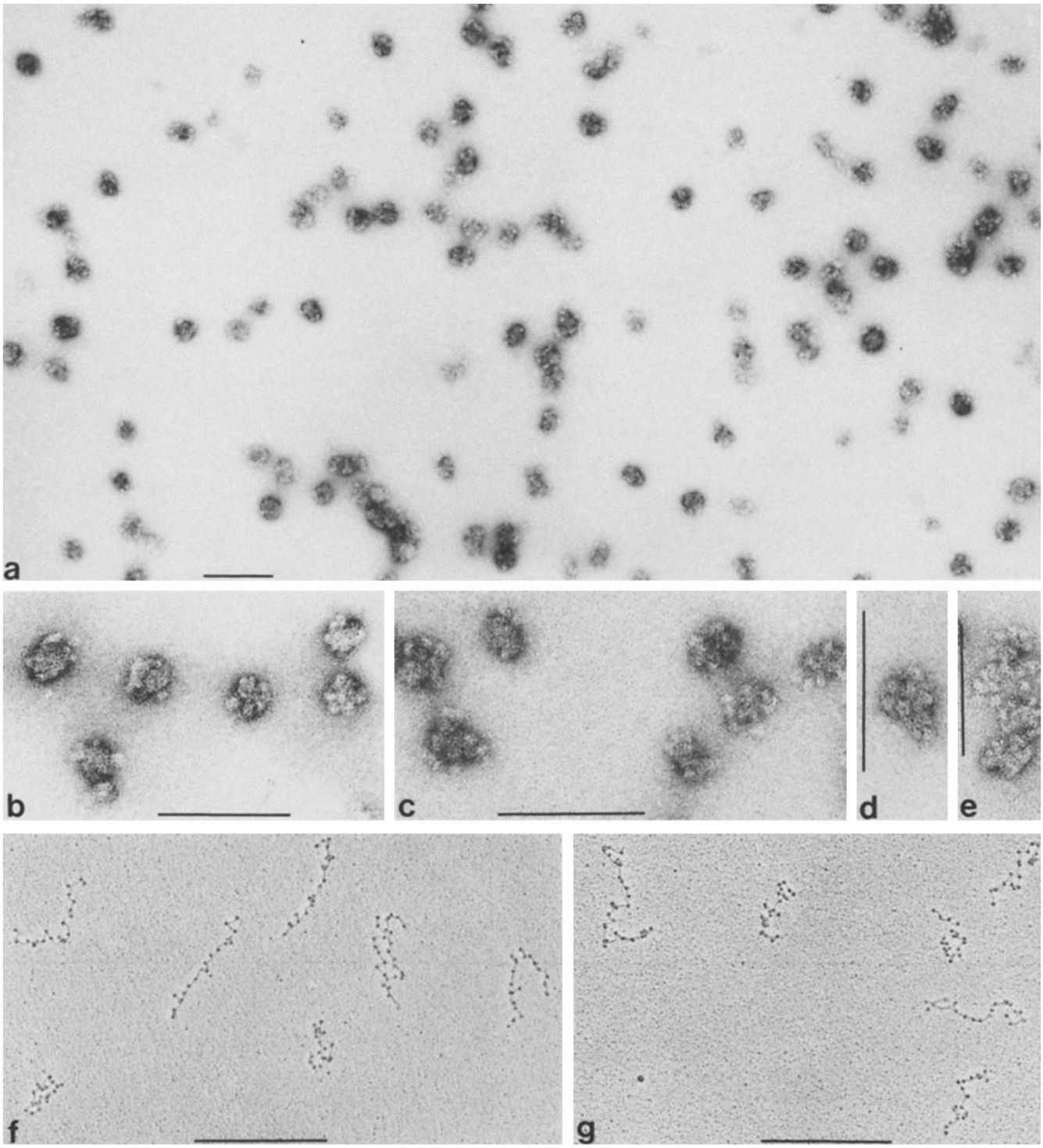

FIGURE 6 Compact and extended configuration of nucleofilaments of supranucleosomal chromatin particles from chicken erythrocytes. When chromatin of peak fractions nos. 9–11 (see Fig. 5) is spread with aldehyde fixation but in the presence of 100 mM NaCl and then negatively stained with uranyl acetate, the compact globular shape of the 35-nm particles is well preserved (*a*). Higher magnification reveals internal nucleosomal substructures (*b–e* show a sequence of different forms of unfolded nucleosomal arrays). Incubation in low salt buffer results in unraveling of the compart particles into extended nucleofilaments containing an average of 20 nucleosomes (*f*). These extended nucleosomal chains are also seen when the 35-nm particles are dialyzed against low salt buffers and rerun in sucrose gradients made up in low salt buffer (*g*, for preparative conditions see text). Bars, 1.0 μm (*a*); 0.1 μm (*b–e*); and 0.5 μm (*f* and *g*). (*a*) × 111,000; (*b*) × 219,000; (c) × 250,000; (*d*) × 275,000; (e) × 219,000; (*f* and *g*) × 43,000.

of these subunit particles obtained from hepatocytes, avian erythrocytes, and sea urchin spermatozoa argues against their origin as a preparative artifact or as a uniformly sized "detached turn" fragment, which would be predicted from the model of a universal solenoid of Finch and Klug (15; cf. 4, 7).

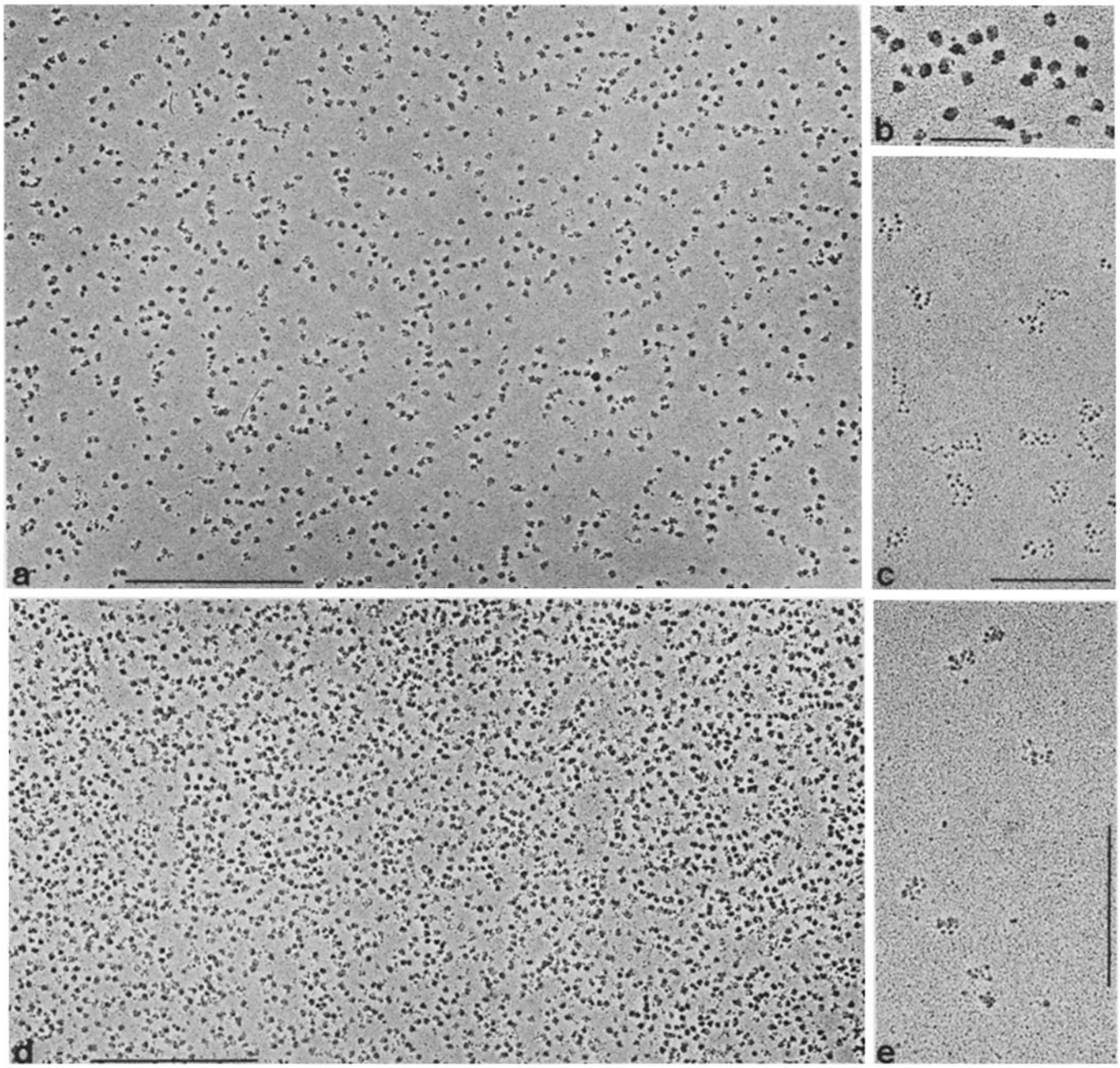

FIGURE 7 Supranucleosomal chromatin particles (pooled fractions nos. 4–6) from chicken liver (*a–c*) and rat liver (*d* and *e*) in compact and unraveled form. In spread preparations the higher order chromatin subunits isolated from rat (*a*) and chicken (*d*) liver appear as uniformly sized 32-nm granules (at higher magnification in *b*). On incubation in buffers of low ionic strength prior to the spreading procedure the compact particles unravel into chains of nucleosomes mostly containing eight nucleosomes (*c*, rat liver; e, chicken liver). Bars, 1 μm (*a* and *d*); 0.2 μm (*b*); and 0.5 μm (*c* and *e*). × 28,000 (*a* and *d*); × 59,000 (*b*); × 38,000 (*c*); × 52,000 (*e*).

Our observations suggest that the higher order organization of the nucleofilament differs among the different types of nuclei examined. The exceptionally large diameter of the subunits dissected from chromatin fibrils of sea urchin sperm heads corresponds with measurements of fibril widths in sections through fixed intact spermatids and spermatozoa of this and other sea urchin species (29, 66; this study). Moreover, the diameter of the isolated sea urchin chromatin particles is identical with that of the "superbead" structures described in chromatin spread after brief exposure to low salt concentrations (66). Therefore, the difference in diameter and nucleosome content between chromatin particles obtained from sea urchin sperm chromatin and those from avian erythrocytes and avian as well as mammalian liver appears to be related to differences in widths of chromatin fibrils of the intact cell, indicating true differences in higher order organization of chromatin in the cells.

Nonrandom cleavage of chicken erythrocyte chromatin by micrococcal nuclease at near physiological strength has also been observed by other authors (39, 42). The size of our 105

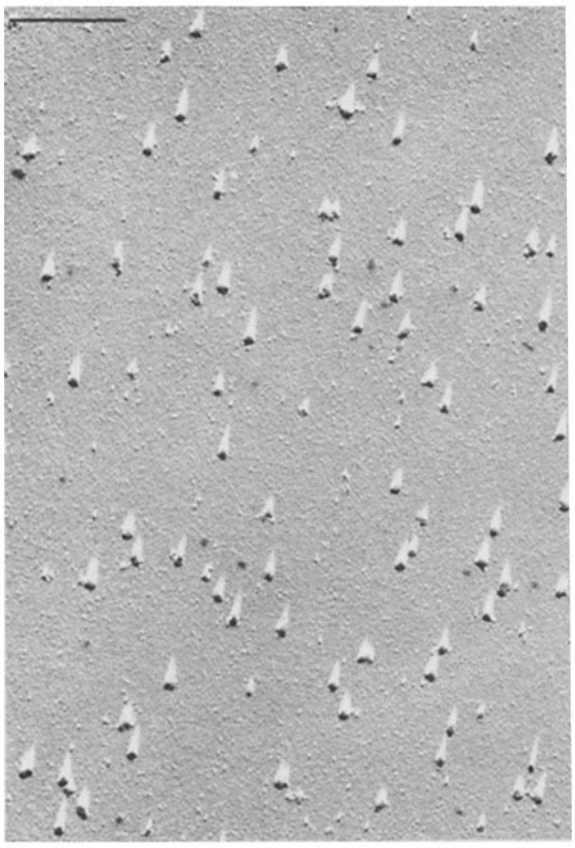

FIGURE 8 Unidirectional metal-shadowing (8°) of supranucleosomal chromatin particles from chicken erythrocytes (pooled peak fractions 8–12), showing the predominance of shadow lengths corresponding to the isodiametric shape of the large particles. Note also the occurrence of some smaller particles, down to the nucleosomal size. Bar, 1.5 μm. × 30,000.

S chromatin particles isolated in high yield from chicken erythrocyte nuclei corresponds to the mean diameter of the "unit chromatin thread" determined in sections through nuclei of fixed erythrocytes (13, 60). Indeed the correspondence is greater if one corrects for shrinkage during dehydration and embedding (cf. 28), and to the low-angle X-ray diffraction peak at 40 nm observed in living erythrocytes (27, 28). The diameter of the isolated particle is also similar to the values reported for repeating granular subunits in chromatin fibrils of nuclei lysed at reduced ionic strength (32 nm, reference 32; 33 ± 6 nm, reference 39; 29 ± 3 nm, reference 67; 34 nm, reference 3). Thus, under all conditions of preparation sea urchin sperm chromatin fibrils and higher order particles derived therefrom are larger than those of chicken erythrocyte chromatin.

Our values determined for the supranucleosomal chromatin subunits from rat liver confirm the data of Strätling et al. (52) and Kiryanov et al. (25) and, in view of the predominance of histones in these particles (Fig. 11), we prefer not to defend again our conclusion that these particles are chromatin and not ribonucleoprotein (see Introduction). The finding that chicken liver chromatin gives rise to a chromatin particle practically indistinguishable from that isolated from rat liver chromatin but clearly different from the particles isolated from chicken erythrocytes suggests that the differences observed are not due to species differences but are related to cell differentiation. Comparison with data available in the literature suggests that the supranucleosomal arrangement of HeLa cells (8) and lymphocytes (20, 42) is similar to that of hepatocytes rather than to that present in avian erythrocytes.

This study also supports the concept of an organization of the higher order subunits into beaded chains with a distinct and uniform mean size of the monomeric beads that is characteristic for the specific kind of chromatin. We have noticed such oligomeric chains not only after centrifugation of unfixed particles on electron microscopic grids but also after fixation of the particles in suspension and we have not found extended chains of beads in peak fractions. Therefore, an artificial aggregation into linear arrays as described by Jorcano et al. (22), seems to be excluded even at very high particle concentrations.

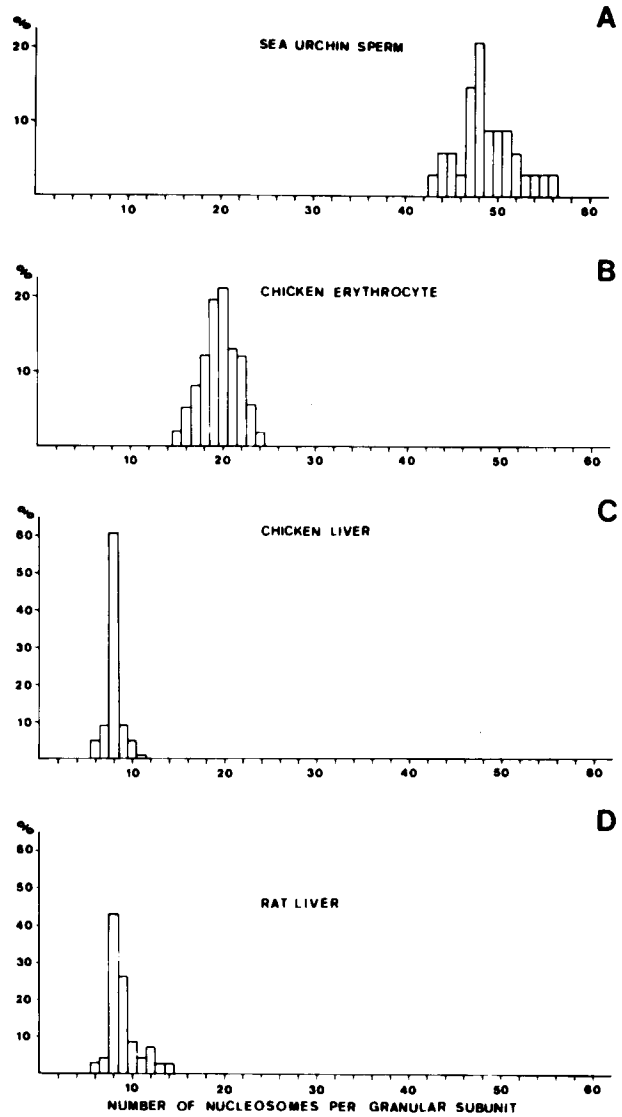

FIGURE 9 Numbers of nucleosomes per granular supranucleosomal subunit in different types of chromatin. Higher order chromatin particles were unfolded by dilution into buffers of low ionic strength, spread for electron microscopy, and nucleosomes per nucleofilament unit were counted. Each type of chromatin shows a distribution with a significant maximum specific for this type of chromatin. The mean values for nucleosomal contents have been determined to be 48 for sea urchin sperm (*A*, $n = 150$), 20 for chicken erythrocyte (*B*, $n = 450$), and 8 for chicken (*C*, $n = 150$) and rat (*D*, $n = 150$) liver.

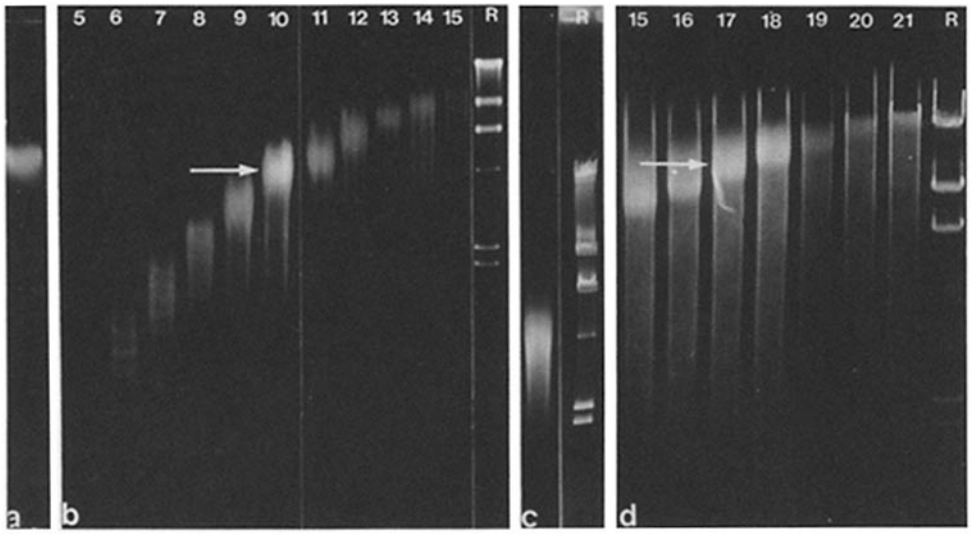

FIGURE 10 Agarose gel electrophoresis of higher order chromatin particles and size determinations of DNA contained in supranucleosomal chromatin particles from chicken erythrocytes and sea urchin sperm. Chicken erythrocyte chromatin fractions from the peak region of a sucrose gradient were pooled and aliquots were analyzed by electrophoresis on 0.3% agarose (*a*). Note migration of particles as a single band.

DNA of supranucleosomal particles from chicken erythrocytes (*b* and *c*) and sea urchin sperm cells (*d*) was extracted and analyzed by electrophoresis on 1% agarose. (*b*) Chicken DNA samples of fractions no. 5–15 from a sucrose gradient centrifugation similar to that shown in Fig. 2*B* are shown in slots 5–15 (arrow denotes peak fraction with maximal DNA content). (*c*) Chromatin particles of fraction no. 10 were dialyzed against low salt buffer and re-run in a sucrose gradient made up in low salt buffer. Note that the particle DNA (extended electrophoresis, left slot) is still approximately 4.5 kb long. (*d*) Sea urchin DNA of particle fractions no. 15–21 from a separation similar to that shown in Fig. 2*A* is shown in slots 15–21 (arrow denotes peak fraction). Reference slots (*R*) contain *Hind III* restriction fragments of λ-DNA (kilobase values are indicated on right margin).

In view of the data presented in Table I we conclude that the density of nucleosome packing and the mode of nucleofilament arrangement are different in the different cell types examined. Differences of higher order chromatin organization in different cell types have also been mentioned in X-ray diffraction studies (27, 28), but were difficult to interpret. We are fully aware, however, that the notion of such pronounced cell type-specific differences of supranucleosomal organization of chromatin is contrary to the concept of a universal solenoid of six nucleosomes per turn and is also at variance with the conclusions of a recent study by McGhee et al. (31) using chromatin particles dialyzed overnight against 0.25 mM EDTA. At present, we are not in a position to explain these differences but we suspect that they are due to different preparations of chromatin.

The molecular basis for the different modes of supranucleosomal organization in the three different cell types compared in this study is not understood. It is interesting to note that all three cell types express different patterns of histones, in particular in the H1/H5 families that are known to promote the higher order packing of nucleosomes (1, 23, 42, 58). Modulation of the nucleosome structure by differences of histone composition has been reported for sea urchin spermatozoa and embryos (48, 49). It will be interesting to experimentally exchange the H1/H5 histones and examine the specific reconstituted supranucleosomal particles (for reconstitution of correctly-sized sea urchin and erythrocyte chromatin in vitro see 66, 67).

Most of the direct physical data on the higher order organization of native chromatin can be explained by both the solenoidal fibril and the nucleomeric fibril ("superbead") model. In our opinion, data obtained from chromatin solubilized by prolonged exposure to low salt conditions are not necessarily pertinent to discussions of the organization of chromatin in situ, unless the restoration of the specific higher order structure has been proven. Clearly, modular or beaded regions can be detected over a certain range of ionic strength, including 100 mM NaCl sections, even in most of the micrographs published to show solenoidal arrangements (e.g., 2, 4, 7, 15, 26, 58), obviously pointing to the vulnerability of interpretations of morphological features to subjective selections. Limited helical regions of zig-zag arrays or parallel rows of nucleosomes, suggestive of deranged helical coils, have been noted by previous authors studying either chromatin briefly exposed to low salt buffer or low salt treated, fragmented chromatin that has reorganized into higher order structure in vitro (e.g., 41, 58, 67). We explain these structures as intermediate configurations of chromatin fibril unraveling (67).

An important feature of the solenoidal model is the central hole which in the 30–35-nm fibrils would be 10–15 nm wide (15, 53). Such a hollow core has not yet been seen, not even in the thick chromatin fibrils of sea urchin sperm where it would be expected to be ~25 nm in diameter, i.e., close to the size of a ribosome. Even if one assumes that most of the internucleosomal "spacer" DNA (which is more in the case of the sea urchin sperm than in other kind of chromatin; cf. 31, 50) to be located in this central cavity (15, 51), one has to expect a remaining difference of mass density and staining in this central region. Moreover, we find it very difficult to construct a regular solenoid of 48 nm outer diameter containing six nucleosomes per turn (cf. 15, 31). Obviously, the present information on higher order organization of chromatin is still insufficient to reach a final decision as to the arrangement of the nucleofilament. We hope that the preparation of defined subunits from native chromatin described in this study will contribute to the elucidation of the structural principles governing the accomodation of chromatin in the living cell, including the possible changes of chromatin organization that may occur during certain processes of cell differentiation.

We thank Dr. C. Petzelt (this center) for providing the sea urchins and Drs. A. Alonso, J. L. Jorcano, U. Scheer, G. Krohne, J. Kleinschmidt, and U. Müller for valuable discussions. We thank Miss M. Schrenk, Mr. C.-T. Bock, and Mrs. C. Grund and S. Mähler for

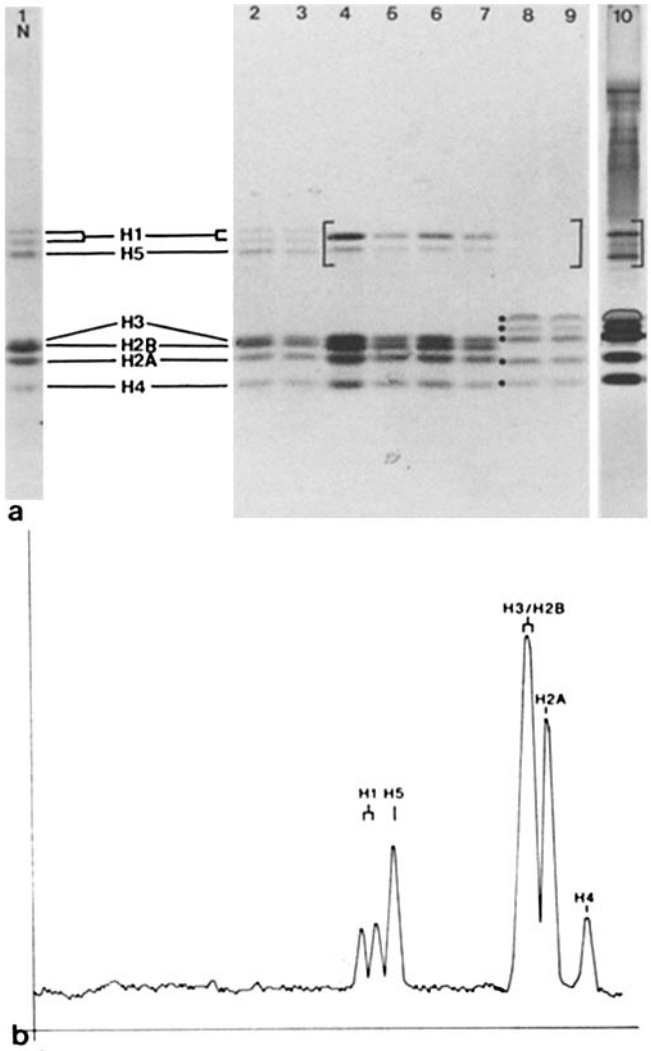

FIGURE 11 Polypeptides of higher order chromatin particles demonstrate purity and integrity. Proteins of whole chicken erythrocyte nuclei (designated *N* in *a*, lane *1*) and chromatin particles (peak fractions) isolated in sucrose gradients were analyzed by SDS-PAGE and visualized by Coomassie Blue-staining (*a*, lanes *2*–*9*). Note the presence of all histones, including histones H1/H5 in chicken erythrocytes (lanes *2* and *3*), histones H1 (brackets: rat liver, lanes *4* and *5*; chicken liver, lanes *6* and *7*) and the sperm-specific histones of sea urchins (lanes *8* and *9*; the sperm-specific H1 histones, brackets, have been only weakly stained by Coomassie Blue under the conditions used here, lanes *8* and *9*, but are well seen after extensive silver staining in lane *10*). Dots in lane *8* denote the nucleosomal core histones of sea urchin sperm cells (from top to bottom: $H2B_1$ and $H2B_2$, *H3, H2A, H4*). Note that the relative proportion of histone H5 is similar in total chromatin of isolated nuclei (lane *1*) and in the supranucleosomal particles (lane *2*). (*b*) Densitometer tracing of lane *2* in *a*. It is important to note that the specific Coomassie Blue-staining used (Serva, Heidelberg, Federal Republic of Germany) did not stain histones H1, H4, and H5 as effectively as other Coomassie Blue preparations.

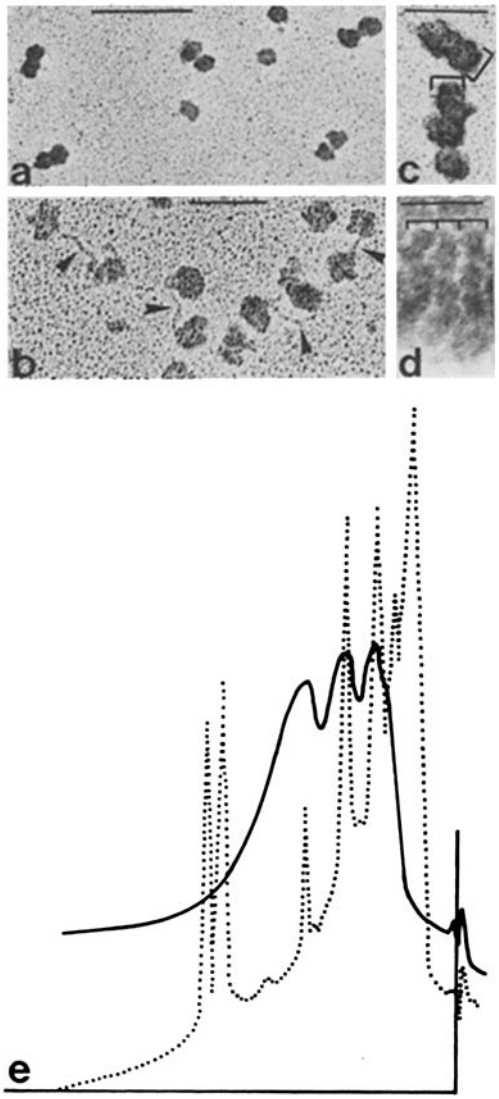

FIGURE 12 Oligomers of higher order chromatin particles from chicken erythrocytes as revealed by electron microscopy of spread preparations (*a*–*c*) and by DNA analysis (e). Spread preparations made from fractions no. 14–16 (cf. Fig. 2*B*) show pairs of higher order particles (*a*; arrowheads in *b* denote interglobular DNA), whereas heavier material is enriched in chains of 3, 4, and more of these particles (*c*; fractions no. 17–20). For comparison, the appearance of chromatin fibrils, in sections grazing to the nuclear periphery, in nuclei of fixed intact cells is shown (*d*). Brackets in c and *d* denote the knobby higher order chromatin fibrils in spread preparation (c) and in section (*d*). Bars denote 0.1 (*b*–*d*) and 0.2 μm (a). × 65,000 (*a*); × 100,000 (*b*); × 110,000 (*c*); × 110,000 (*d*). Sizes of DNA contained in such oligomer-enriched fractions were determined by gel electrophoresis on 1% agarose pooled fractions no. 12–18 and analyzed by densitometer tracing (e). Major peaks of DNA sizes appear at 4.3, 6.1, and 9.5 kb, respectively (solid line in e). For comparison *Hind III*-restriction fragments of λ-DNA are shown (dotted line in *e*; kilobase values are given in Fig. 10).

skillful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (grant Ze 184/2-2).

Received for publication 14 November 1983, and in revised form 9 March 1984.

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