ULTRASTRUCTURAL FEATURES OF CHROMATIN ν BODIES

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There is increasing evidence that chromatin fibers consist of linear arrays of spheroid nucleohistone particles, the ν or "nu" bodies (7, 12, 15-19, 21, 27, 33, 34). More than any previous conception of chromatin (2, 22-24), this model is supported by: (a) biochemical studies of isolated ν bodies (3, 8, 14, 19-21, 25-27, 29, 31); (b) neutron diffraction studies of chromatin (1); and (c) data on the retention of low-angle X-ray reflections during the processing of chromatin for electron microscopy (18). Furthermore, a particulate model of chromatin furnishes a basis for interpreting the histone-to-histone interaction data (4-6, 9-11, 13, 17, 28, 30). In addition, theoretical considerations of the periodicities of close-packed arrays of ν bodies form a basis for explaining the low-angle X-ray reflections of chromatin.[1] The present study, employing high-resolution electron microscopy, describes several characteristics of ν-body structure and of the relationship of ν bodies to the connecting strands.

MATERIALS AND METHODS

Preparation of Nuclei

Chicken erythrocyte nuclei were isolated, as previously described (16-18), for: visualization of freshly spread nuclei (16, 18); isolation of chromatin fragments by sonication (19, 27); and preparation of soluble and reassociated chromatin. For preparation of nuclease-resistant chromatin fragments, nuclei were isolated by a different procedure,[2] modified from published methods

(3, 8, 14, 31, 32). Red cells were lysed and washed in a buffer of 10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris (pH 7.4), and 0.5% Nonidet P-40 (Particle Data Inc., Elmhurst, Ill.). Nuclei prepared by this rapid procedure exhibited linear arrays of ν bodies. Spreads of isolated nuclei were prepared as usual (16, 18) except that the grids were stained and dried in 5 mM aqueous uranyl acetate.

Preparation of Soluble and Reassociated Chromatin

Soluble nucleohistone was prepared by established procedures (18). Dissociation of the histones and DNA was accomplished by overnight dialysis of nucleohistone ($A_{260} \sim 3.4$) against 2 M NaCl, 0.001 M sodium cacodylate (pH 7.0) at 4°C. Reassociated chromatin was prepared by dialysis against buffers containing decreasing concentrations of NaCl (18) but not containing urea. Erythrocyte histones were associated with heterologous DNA (Escherichia coli, T_7, and T_4) via the following scheme: chromatin in 2 M sodium chloride was centrifuged overnight (16-18 h) at 63,000 rpm in a Spinco Ti-65 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), and the histone supernate recovered. Heterologous DNA, dissolved in 2 M NaCl, was added to the total histones, increasing the A_{260} to the initial value of the chromatin before centrifugation. In a separate experiment, purified chicken DNA was added to the histone supernate. All DNA-histone mixtures were dialyzed successively against decreasing NaCl concentrations, and against 0.0025 M sodium ethylenediaminetetraacetic acid (EDTA), followed by dialysis against 0.001 M sodium cacodylate buffer (pH 7.0). Native and reassociated chromatins were centrifuged onto a carbon grid through a cushion of 10% formaldehyde (pH 7.2), washed with dilute Photo-Flo (pH 7.0) (Eastman Kodak Co., Rochester, N.Y.), dried, and stained and dried in 5 mM aqueous uranyl acetate.

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[1] Carlson, R. D., and D. E. Olins. 1975. Chromatin model calculations: arrays of spherical ν bodies. Submitted for publication.

[2] Senior, M. B., J. P. Breillatt, R. D. Carlson, A. L. Olins, and D. E. Olins. 1975. Properties of nuclease-derived chromatin ν bodies. Manuscript in preparation.

Preparation of Sonicated Chromatin Fragments

Water-swollen erythrocyte nuclei were fixed with formaldehyde, sonicated, and fractionated by sucrose gradient ultracentrifugation as described previously (19, 27). Fractions enriched with monomer fragments ($A_{260} \sim$ 1-2) were applied to a glowed, carbon-coated grid, washed with Photo-Flo, dried, and stained and dried in 5 mM uranyl acetate.

Preparation of Nuclease-Resistant Chromatin Fragments

Erythrocyte nuclei were diluted to a concentration of $\sim 1.4 \times 10^9$ per ml, and made 10^{-4} M in $CaCl_2$. Micrococcal nuclease (Worthington lot no. 54S654, Worthington Biochemical Corp., Freehold, N.J.) was added to a final concentration of ~ 60 μg of enzyme per ml. Samples were incubated 1 h at 37°C. Digestion was terminated by the addition of sodium EDTA to 40 mM and cooling to 4°C. After sonication for 2-3 min, nuclease-resistant fragments were pelleted by centrifugation for 10 min at ~ 4000 g and resuspended in 0.2 mM sodium EDTA ($A_{260} \sim 50$-100). Aliquots (0.1-0.2 ml) were loaded on a 5-20% sucrose gradient (containing 0.2 mM sodium EDTA, pH 7.0) and centrifuged for 12 h at 35,000 rpm in a Spinco SW-41 rotor. For electron microscope visualization, one drop of the unfixed monomer particles (made 0.5 mM $MgCl_2$) was applied to a carbon-coated grid, stained and dried in 5 mM aqueous uranyl acetate. No fixation or drying from Photo-Flo was employed.

RESULTS

Freshly isolated chicken erythrocyte nuclei, swollen with 1 mM sodium EDTA (pH 7.0), spread as described previously (16, 18) and stained with aqueous 5 mM uranyl acetate, displayed the usual "beads-on-a-string" morphology. At high magnification (Fig. 1 a and b) two reproducible structural features could be observed: (a) ν bodies frequently displayed a lateral association with the connecting strand (thick arrowheads), although many other ν bodies appeared to be attached across a diameter of the spheroid particle; and (b) some ν bodies displayed a spot of stain near the center of the particle (thin arrows).

Soluble and reassociated chromatin preparations displayed these same ultrastructural features. Many ν bodies exhibited central staining and lateral attachment to the connecting strands in soluble erythrocyte chromatin, reassociated erythrocyte chromatin, and erythrocyte histones associated with chicken DNA, E. coli DNA, and T_7 and T_4 (Fig. 1 c) bacteriophage DNAs. However, the lengths of the connecting strands were considerably more variable in preparations of soluble and reassociated chromatin, compared to spreads of freshly isolated nuclei.

Chromatin fragments prepared by sonication were fractionated by sucrose-gradient ultracentrifugation (Fig. 2 a). Fractions enriched with monomer fragments frequently revealed a "tadpole" appearance with a single ν body attached to a portion of connecting strand (Fig. 1 d-f). Occasionally, "double-tailed" fragments and ν body dimers were observed. These observations are consistent with possible double-strand breaks at the junction of the ν body and the connecting strand. Many particles exhibited the central staining described earlier (thin arrows).

Chicken erythrocyte nuclei were digested with micrococcal nuclease and fractionated by sucrose-gradient ultracentrifugation (Fig. 2 a). Multiple sedimentation peaks were observed, in agreement with the findings of other workers (14, 20). The slowest sedimenting peak proved to be predominantly monomer ν bodies (Fig. 2 b). Almost all of these monomer fragments were devoid of tails. Most of the particles showed central staining (Figs. 2 c and 3) and exhibited a mean diameter and standard deviation of 81 $\pm$ 8 Å (the diameter was measured between the outer staining edges). The central staining spot had a mean diameter of 15 Å, or about one fifth the total particle diameter. Occasionally, particles were observed with a line of central staining partially or completely crossing the ν body at its diameter.

DISCUSSION

The use of dilute aqueous uranyl acetate stain has permitted us to better visualize structural features of spread chromatin fibers and isolated chromatin fragments than has been possible in previous studies (16, 18). The electron micrographs show some variation in the position of the ν body with respect to the connecting strand, i.e., some ν bodies show a lateral attachment while others are connected across their diameter. Frequently the presence of a central stained area within a ν body can be observed. These variations in morphology could arise from either different classes of particles or variations induced by the microscopy techniques.

Variation in ν-body position relative to the connecting strand could represent different views of a laterally attached ν body. The length of the connecting strand can be extremely variable (16,
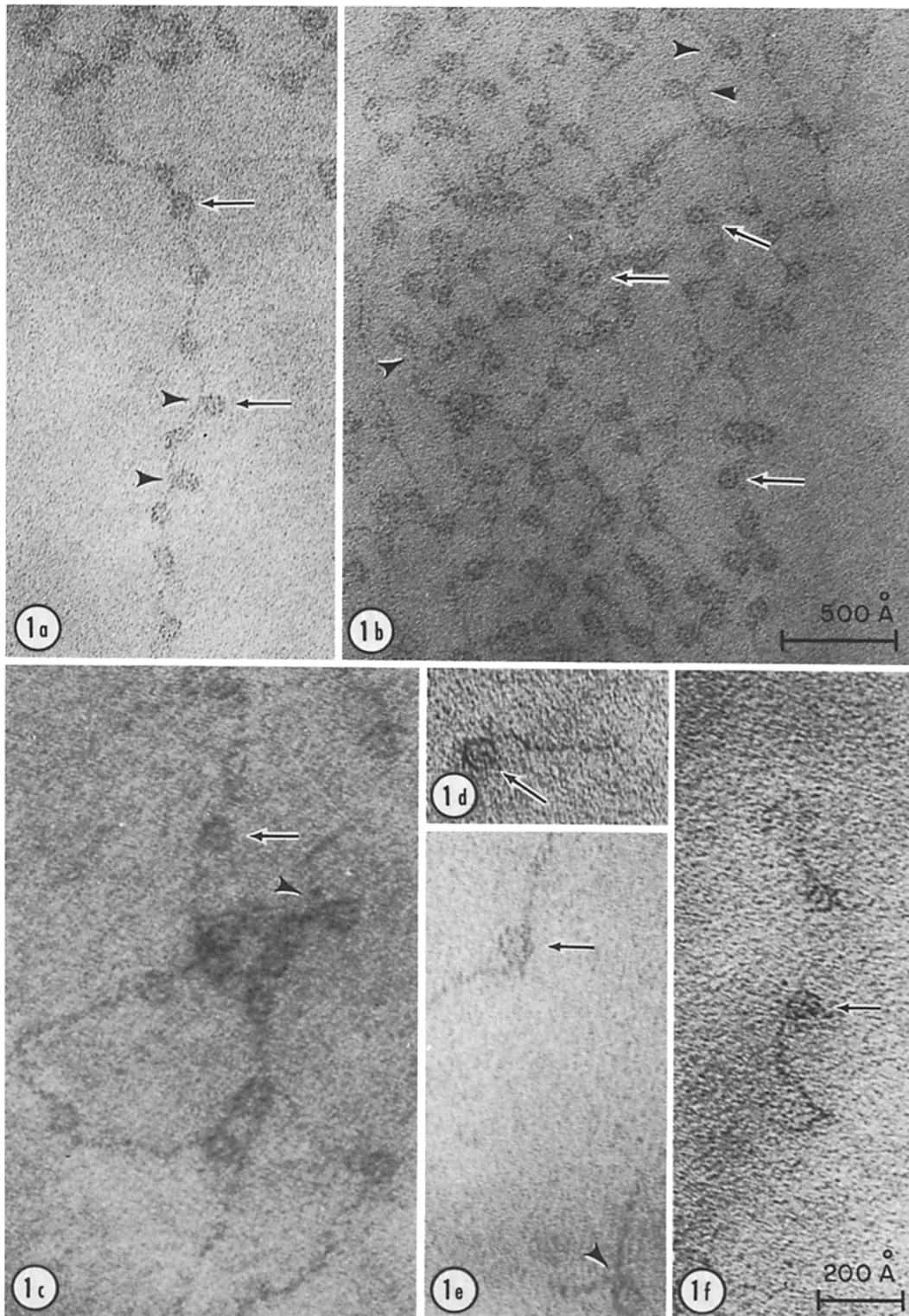
FIGURE 1 (*a* and *b*) Electron micrographs of uranyl-stained spreads of chromatin fibers from isolated chicken erythrocyte nuclei, (*c*) an associated complex of erythrocyte histones and T_4 DNA, and (*d–f*) "monomer" fragments obtained from sonication of formaldehyde-fixed erythrocyte nuclei. Thin arrows denote ν bodies showing a central spot of stain; thick arrowheads denote ν bodies with lateral association to the connecting strand. Magnifications: *a* and *b*, × 357,000; *c–f*, × 626,000.
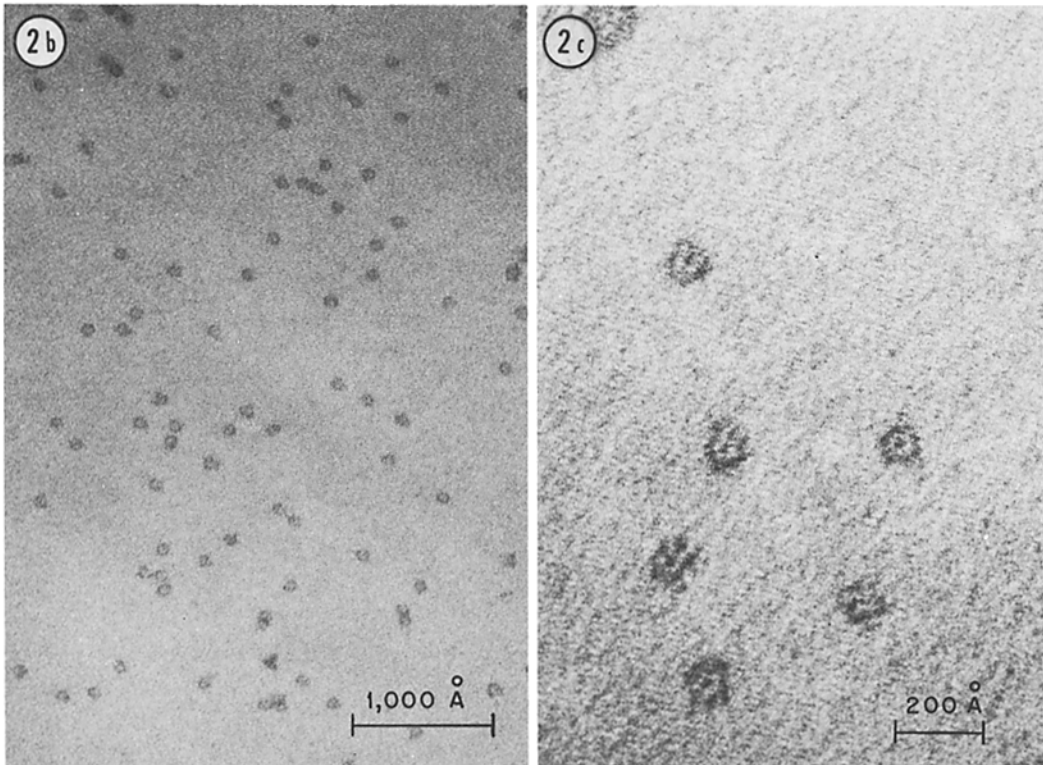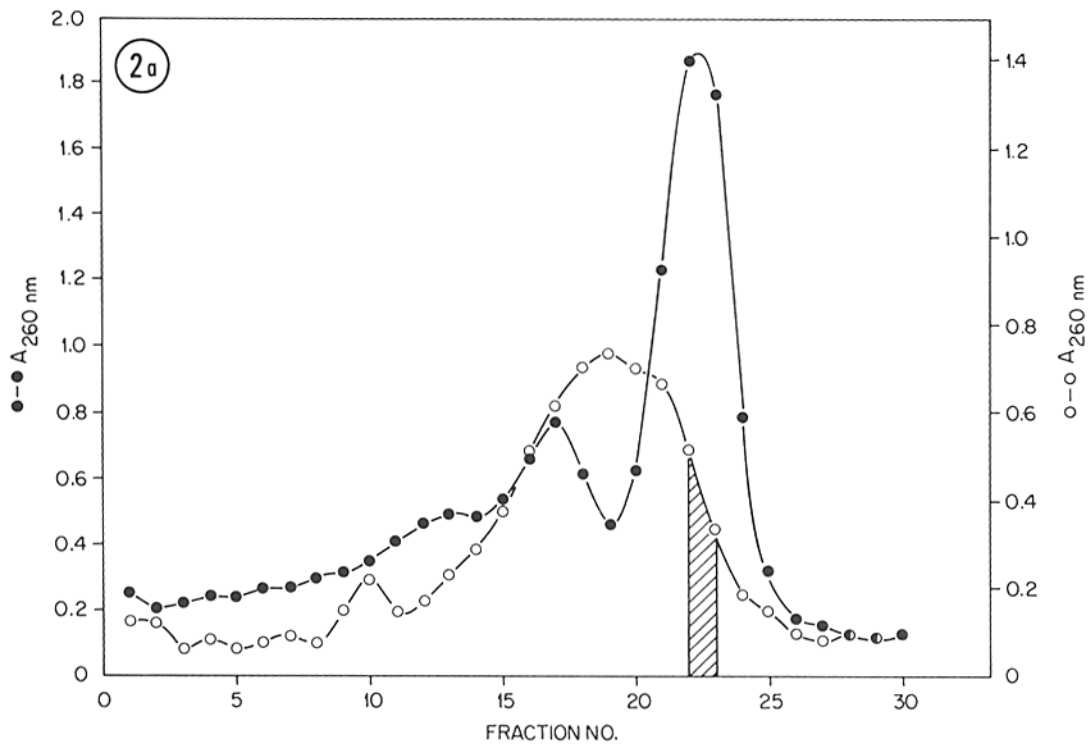
FIGURE 2 (*a*) Comparison of sucrose gradient profiles of sonicated, fixed chromatin (O——O) and nuclease-digested chromatin (●——●). The shaded area corresponds to the region of the gradient enriched with monomer fragments produced by sonication. (*b* and *c*) Electron micrographs of monomer *ν* bodies obtained after micrococcal nuclease digestion and fractionation by sucrose gradient ultracentrifugation. Magnifications: *b*, × 193,000; *c*, × 600,000.
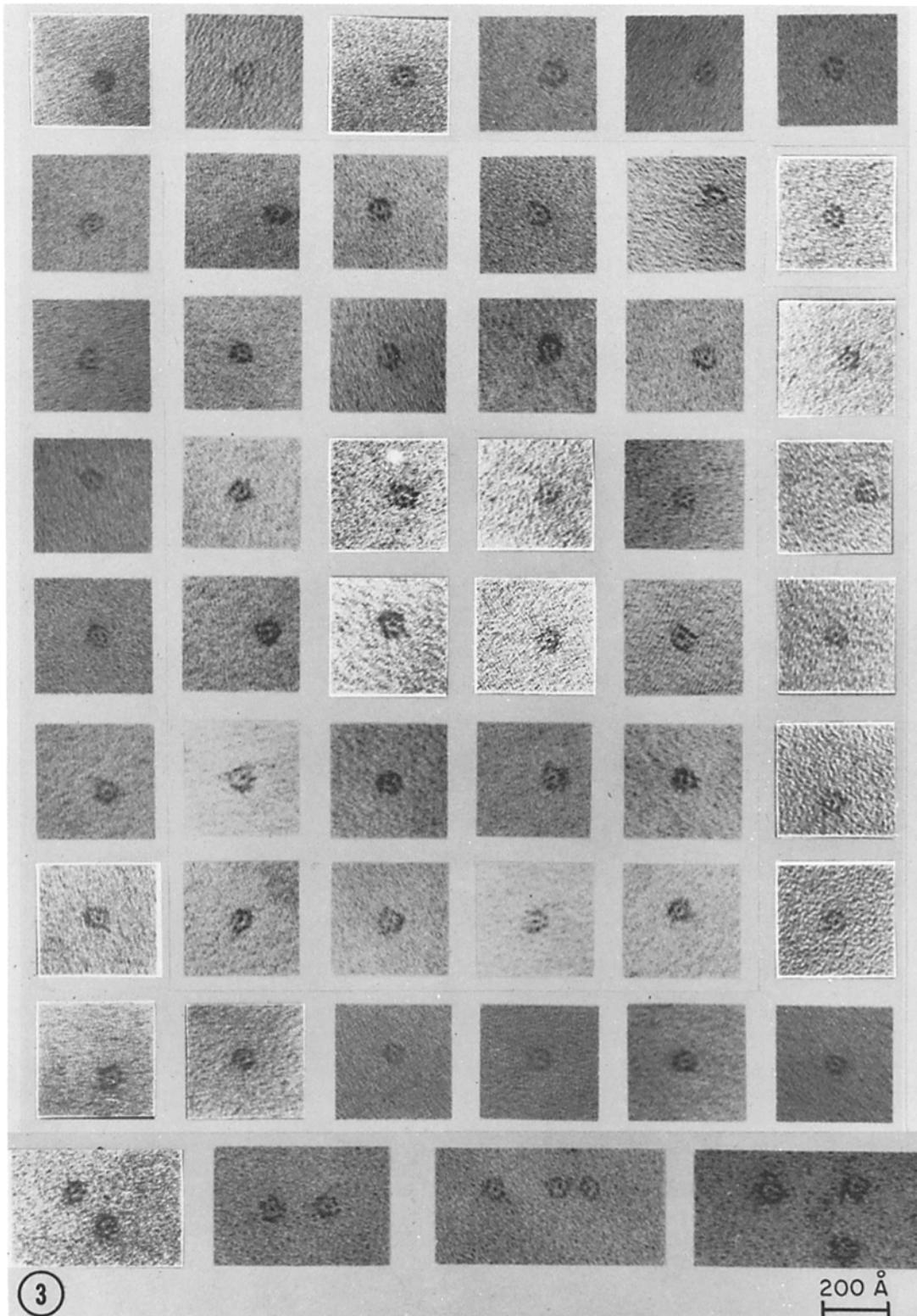
FIGURE 3   Electron micrographs of monomer ν bodies obtained after digestion with micrococcal nuclease. Particles have been chosen that exhibit clear central staining. × 580,000.

18, 21), indicating that it may represent the stretching of less tightly associated DNA from the ν body. However, employing uranyl acetate in methanol as a stain (see Fig. 6 of reference 7) we have obtained our clearest visualization of connecting strands of approximately constant length.[3] In these preparations of spreads of freshly isolated chicken erythrocyte nuclei the connecting strands had a mean length of ~140 Å, which could correspond to 40–50 nucleotide pairs of DNA.

The significance of the central staining is not yet clear. It could represent a hole passing through the center of a ν body, consistent with suggestions by others (12, 34). Although we are employing a negative staining technique with aqueous uranyl acetate, the uranyl ion generally shows a preferential binding to nucleic acids (35). Thus, it is conceivable that the 15 Å spot represents a DNA molecule within the center of a ν body. It is clear, however, that this spot does not arise from fragmentation of the chromatin by sonication or nuclease digestion, since it can also be observed in nuclear spreads. By employing dark-field microscopy, other types of stains, and a tilting stage, we hope to make a more definitive statement concerning the internal structure of a ν body. Preliminary studies employing 1% phosphotungstic acid (pH 7.0) as a negative stain also reveal central staining within purified monomer ν bodies. The present observation that heterologous DNA can associate with histones, resulting in formation of ν bodies, is consistent with other evidence (21), and suggests that specific nucleotide sequences are not essential to ν-body structure.

SUMMARY

Spread chromatin fibers and isolated chromatin fragments prepared from chicken erythrocyte nuclei were stained with dilute aqueous uranyl acetate. High-resolution electron micrographs reveal two new morphological features exhibited by many of the chromatin ν bodies: (a) lateral association of the ν body with the connecting strand, and (b) a centrally stained spot ~15 Å wide, possibly corresponding to a hole or crevice within the ν body.

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[3] Olins, A. L., and D. E. Olins. Unpublished observations.

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