(Cornell Univ. Press, New York, in press)]. Similarly, TYA may lack significant effects on platelet aggregation in vivo.
18. Agents used to induce platelet aggregation were adenosine diphosphate (Sigma), epinephrine (adrenaline chloride: Parke-Davis), bovine thrombin (Parke-Davis), and saline extract of human subcutaneous connective tissue (*8*).
19. Supported in part by National Heart and Lung Institute grant HE 14595. We thank J. Rogers (Roosevelt Hospital) for technical assistance.

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Spheroid Chromatin Units (*ν* Bodies)

Abstract. *Linear arrays of spherical chromatin particles (ν bodies) about 70 angstroms in diameter have been observed in preparations of isolated eukaryotic nuclei swollen in water, centrifuged onto carbon films, and positively or negatively stained. These bodies have been found in isolated rat thymus, rat liver, and chicken erythrocyte nuclei. Favorable views also reveal connecting strands about 15 angstroms wide between adjacent particles.*

The packaging of DNA within eukaryotic chromosomes continues to be a formidable structural problem. Packing ratios greater than 100/1 (DNA length/chromatid length) are not uncommon for metaphase chromosomes (*1*). The DNA concentrations within localized regions of interphase nuclei may approximate 200 mg/ml or more (*2*). Acutely aware of this problem, investigators have postulated multiple orders of coiling or folding of a fundamental nucleohistone molecule (*1, 3*). Several models have been derived from low-angle x-ray diffraction studies, including: four DNA molecules packed into a single nucleohistone fibril (*4*); a single DNA double helix and associated proteins folded into an irregular superhelix 80 to 120 Å in diameter and 45 Å in pitch (*5*); and a single DNA-protein fiber constrained into a superhelix 100 Å in diameter and 120 Å in pitch (*6*). Ultrastructural studies have also yielded a profusion of models. Spreading of chromosomes on a Langmuir trough frequently yields fibrils about 250 Å in diameter, although differences due to tissue type, presence of chelating agents, and method of dehydration and drying have been reported (*7*). Direct adsorption of sheared chromatin onto microscope grids has revealed a network of fibers approximately 100 Å wide with numerous side branches 80 to 200 Å in length (*5*). Spraying of chromatin onto a grid yields a network of fibers (*8*) and separated filaments (20 to 30 Å in diameter) containing numerous "nodular" elements about 150 Å in diameter (*9*). Thin sections of nuclei and chromosomes reveal fragments of threads frequently 100 to 200 Å wide (*3, 10, 11*). Bram and Ris (*5*) regard the 250-Å fiber as a folding (or doubling) of a superhelix, due to divalent metal ions, and interpret the thin-section data as artifacts of chelation by buffer ions. Lampert (*12*) views the 250-Å filament as a folding of the superhelix of Pardon and Wilkins (*6*), and explains the thin-section data in terms of shrinkage due to fixation. Despite this divergence of views, there is a consensus that multiple levels of coiling or folding are required to explain the observed variation in chromatin fiber widths.

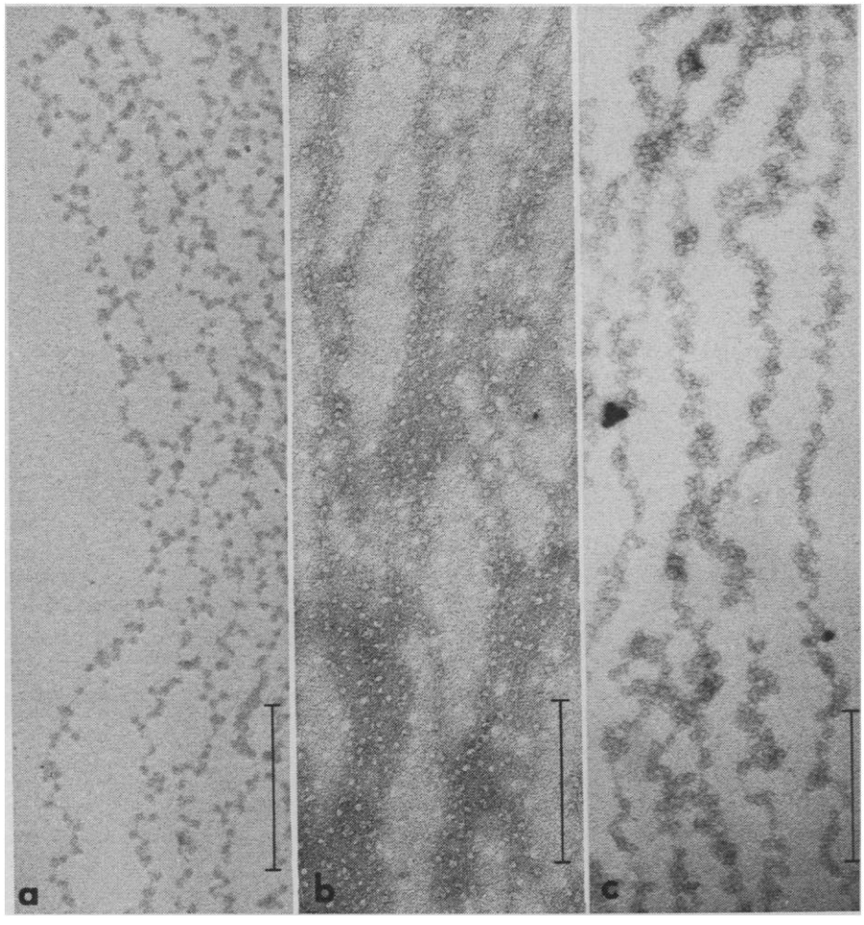

Fig. 1. Chromatin fibers spilling out of ruptured nuclei. The degree of fiber swelling and the proximity of individual *ν* bodies to each other varies within different regions of a single nucleus. Scale bars, 0.2 μm. (a) Rat thymus chromatin, positively stained with a mixture of 4 percent aqueous phosphotungstic acid and 95 percent ethanol (3 : 7), rinsed in 95 percent ethanol, and dried in air. (b) Rat thymus chromatin, negatively stained with 0.5 percent ammonium molybdate, adjusted to *p*H 7.4 to 8.0 with ammonium hydroxide. (c) Chicken erythrocyte chromatin, negatively stained as in (b). Clustering of *ν* bodies is most evident in (c), where groups of three or more are readily visualized. Connecting strands are most easily seen in (b).

We have attempted to visualize chromatin structure by methods different from those cited above. Interphase nuclei were isolated from fresh rat thymus (*13*), rat liver (*2*), and chicken erythrocytes (*2*), washed and centrifuged twice in CKM buffer (*14*) and once in 0.2*M* KCl, suspended in 0.2*M* KCl at a concentration of approximately 10^8 nuclei per milliliter, and diluted 200-fold into distilled H_2O. Nuclei were allowed to swell for 10 to 15 minutes, then made 1 percent in formalin (*p*H 6.8 to 7.0). Fixation proceeded for at least 30 minutes. All operations, up to this point, were at 0°

to 4°C. Aliquots of the swollen and fixed nuclei were centrifuged through 10 percent formalin (*p*H 6.8 to 7.0) onto carbon-covered grids, rinsed in dilute Kodak Photo-Flo, and dried in air, a technique developed by Miller and co-workers (*15*). When examined after positive staining, chromatin fibers could be readily visualized streaming out of ruptured nuclei. Fibers were often very long (about 8 μm in length), unbranched, and in parallel arrays, and revealed irregularly distributed thick and thin regions. Frequently, views of chromatin fibers (Fig. 1a) show spherical particles, ν bodies (*16*), 60 to 80 Å in diameter, connected by thin filaments (about 15 Å wide). Less stretched regions of chromatin revealed apparent packing of ν bodies. Analysis of fiber widths from positively stained preparations showed peaks at 75 to 100, 125 to 150, and possibly 225 to 250 Å (Fig. 2), consistent with the ranges of fiber widths described earlier. Better visualization of the ν bodies and connecting strands has been obtained by the use of negative stains (Fig. 1, b and c). The thickened fiber regions were seen to represent clusters of ν bodies. Measurements of diameters of ν bodies for the different tissues employed yielded the following average diameters and standard deviations: rat thymus, 83 ± 23 Å; rat liver, 60 ± 16 Å; and chicken erythrocyte, 63 ± 19 Å. Connecting strands, for rat thymus chromatin, exhibited average widths of 15 ± 4 Å. Figure 2 demonstrates that the distribution of diameters of ν bodies, measured from negatively stained preparations, superimposes on the lowest peak of fiber diameters calculated from positively stained materials.

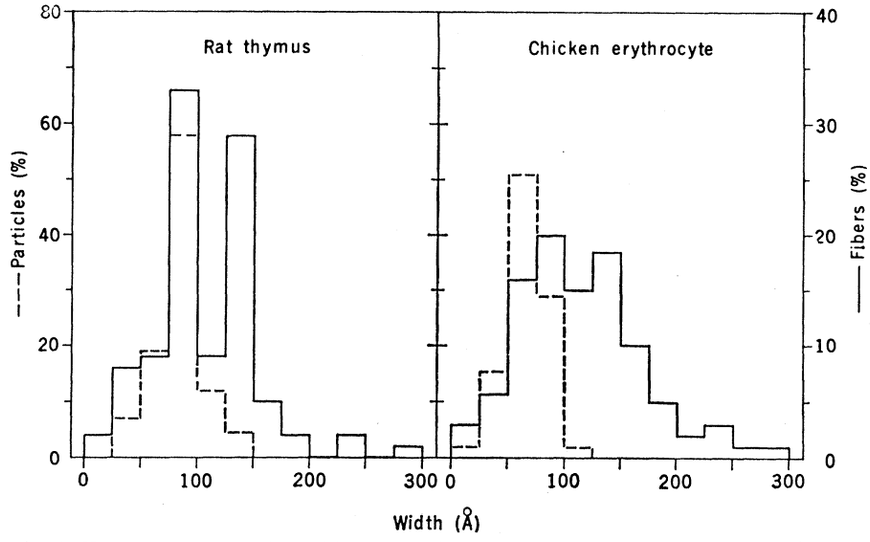

Fig. 2. Histograms comparing widths of positively stained chromatin fibers (solid lines) with diameters of ν bodies (broken lines) from negatively stained preparations. Random sampling of fiber widths was obtained by superimposing a lattice of lines 1 inch apart on the photographic print and measuring the width of any fiber intersecting the grid lines. The positively stained preparations were rat thymus ($N = 100$ samples) and chicken erythrocyte ($N = 360$). The diameters of ν bodies were measured only when their edges were clearly defined and not overlapping another ν body. The preparations for measurements of ν bodies were rat thymus ($N = 114$) and chicken erythrocyte ($N = 200$). A calibration grid (54,864 lines per inch) was photographed with each set of micrographs, printed, and measured simultaneously with the sample photographs.

For a number of reasons we believe that this appearance of chromatin fibers as "particles on a string" is related to the native configuration and is not an artifact of the preparative procedures. Washing of isolated nuclei in CKM buffer (*14*) and in 0.2*M* KCl appears to remove some nonhistone but no histone protein (*17–19*) although histone migration along the DNA cannot be eliminated. However, nuclei so treated reveal the same spectrum of chromatin fiber widths after fixation and thin sectioning (*2*) as those observed for fixed and sectioned whole tissue (*10*). Swelling of nuclei in water leads to stretching and thinning of chromatin fibers, as revealed after fixation in water and thin sectioning (*20*), and the disappearance of several of the low-angle x-ray reflections (*21*). Since addition of divalent metal ions to water-swollen nuclei and chromatin does produce essentially normal low-angle x-ray reflections (*2, 21*) and a partial return of ultrastructural morphology (*20*), the structural changes exhibit some reversibility. Fixation with glutaraldehyde does not markedly perturb low-angle x-ray reflections (*2*), although similar data for formaldehyde are not available. In order to demonstrate that nuclear isolation and washing are not essential for visualization of the chromatin units, fresh whole chicken erythrocytes were disrupted in cold 0.3 percent Joy detergent, followed by swelling, fixation, and centrifugation onto carbon films, a method developed by Miller and Bakken (*22*). Although the spreading of chromatin fibers was not as good as that shown in Fig. 1, particles resembling ν bodies were observed throughout the chromatin.

Assuming that the ν bodies described here represent a real packaging of nucleohistone (*23*), we calculate some of their expected physical properties. For an average particle diameter of 70 Å, a spherical shape, and a partial specific volume of 0.68 cm^3/g (*24*), we estimate an approximate molecular weight of 160,000 per ν body. Further, we assume that every ν body has at least one histone of each of the five classes, and that the sum of their molecular weights is 84,000 (*25*). Therefore, the DNA would have a calculated molecular weight of about 80,000 and a total length of about 400 Å, packed into the spheroid particle 70 Å in diameter. Thus, a packing ratio (DNA length/particle diameter) of about 6/1 might be expected. If there is significant dehydration and shrinkage of the ν bodies, the calculated particle molecular weight would have to be increased. The dimensions of ribosomal particles and subunits, measured by electron microscopy, are roughly half of the calculated hydrated volumes from hydrodynamic measurements (*26*). It would be conceivable, therefore, for each ν body to contain two of each type of histone molecule complexed with a double-stranded DNA with a molecular weight of about 160,000. Further packaging of the DNA might then represent a folded or helical close packing of the spherical ν bodies under the influence of metal cations and noncovalent interaction. Studies should be directed toward fragmentation of chromatin and isolation of particles with properties complementary to the ν bodies.

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16. The Greek letter ν is suggested to denote these spheroid chromatin bodies. This notation was considered to be appropriate since they are both new and nucleohistone. Alternatively, we suggest that they be referred to as "deoxyribosomes" in order to emphasize their particular particulate analogy with ribosomes.
17. Solvents similar to these have been shown to extract ribonucleoprotein particles and nuclear sap proteins [H. Busch, *Histones and Other Nuclear Proteins* (Academic Press, New York, 1965)] including the RNA-containing interchromatin granules (*18, 19*). Furthermore, preliminary observations (D. E. Olins and E. B. Wright, unpublished) indicate that proteins extractable from chicken erythrocyte nuclei by washing in CKM buffer and 0.2*M* KCl contain no detectable histones, as judged by gel electrophoresis in buffers containing sodium dodecylsulfate [K. Weber and M. J. Osborn, *J. Biol. Chem.* **244**, 4406 (1969)].
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23. It is considered unlikely that the ν bodies and connecting strands described in this report might correspond to nuclear ribonucleoprotein particles. Interchromatin granules (200 to 250 Å in diameter) and perichromatin granules (400 to 450 Å in diameter) are considerably larger than the ν bodies (~70 Å in diameter) (*19*). Interchromatin granules would be expected to be extracted during the washes in CKM buffer (*18*). Furthermore, ν bodies are observed in considerably greater numbers than would be expected for the known ribonucleoprotein particles.
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27. A preliminary report of this work was presented at the meeting of the American Society of Cell Biology, Miami, 15 November 1973. At the same meeting, C. L. F. Woodcock reported the observation of similar spherical particles in chromatin fibers. The authors thank O. L. Miller, Jr., and B. A. Hamkalo for advice and criticism and M. Hsie for excellent technical assistance. This work was sponsored, in part, by the Atomic Energy Commission under contract with Union Carbide Corporation, and, in part, by NIGMS grant 1 R01 GM 19334-01 to D.E.O. One of us (D.E.O.) is a recipient of NIGMS research career development award 5 K04 GM 40441-03.

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Tolerance to Heterologous Erythrocytes

Abstract. *Injection of a water-soluble nonantigenic fraction obtained from lysed sheep red blood cells virtually abolishes the subsequent immune response to the red cells. The suppression is systemic and appears to be serum mediated.*

Our understanding of the mechanisms underlying the production of immunological unresponsiveness or tolerance has increased over the past few years, but as yet there is not—and perhaps there cannot be—a unified theory of tolerance that would explain the data obtained from embryonic or neonatal transplantation tolerance experiments, from tumor enhancement and blocking work, from experiments involving disaggregated serum proteins, monomeric flagellin, or pneumococcal polysaccharides; nor may we be able to present a clear rationale for simultaneously understanding both low- and high-zone tolerance, both long-lasting and short-term tolerance, and both antigen- and antibody-mediated tolerance (*1*).

Tolerance to sheep red blood cells (SRBC) has been obtained both in neonatal and adult animals by a prolonged injection schedule with massive doses of SRBC (*2*). More generally, however, unresponsiveness has been induced by treatment with antigen in combination with cytotoxic agents such as cyclophosphamide (*3, 4*). Solubilized SRBC fractions have also been used in induction of tolerance (*5, 6*). There is no agreement concerning the basis for the induced tolerance to SRBC. Depending on the schedule of injections, the number of cells and the source of cells used in restitution experiments, and the timing of assessment of tolerance or of abrogation of tolerance, the lack of response to SRBC has been variously attributed to loss or inactivation of thymus cells, thymus-derived cells, bone marrow or bone marrow–derived cells, or to combinations of these cells (*4*). The possible functioning of suppressor cell populations has also been proposed (*4, 7*).

We have shown (*6*) that a supernatant fraction (after centrifugation at 40,000*g*) obtained after hypotonic lysis of SRBC contains material that can reduce to about 10 percent of normal the subsequent response of adult mice to SRBC. The tolerogenic material did not appear to be immunogenic as measured by induction of direct or indirect plaque-forming cells or by production of serum agglutinins or hemolysins. Reciprocal experiments with hemolyzate preparations of sheep and horse red blood cells indicated, moreover, that the tolerogenic effect of this supernatant is highly specific (*6*).

We now describe experiments designed to characterize the nature of the tolerance to SRBC induced by our supernatant fraction. The experiments show that this tolerance is not associated with a reduction or elimination of immunocompetent cells, but rather that it appears to be the result of a serum-mediated blocking effect.

Sheep red blood cells from individual sheep were purchased (ARS–Sprague-Dawley, Madison); they were washed before use. Tolerogenic preparations

Table 1. Ability of 10^7 spleen cells from normal or tolerant donors to respond to sheep red blood cells after injection into lethally irradiated normal or tolerant syngeneic host animals; PFC, plaque-forming cells; S.E., standard error.

Host	Donor	Experiments (No.)	Animals (No.)	PFC ± S.E. per spleen
Normal	Normal	3	15	4573 ± 882
	Tolerant		15	8878 ± 1666
Normal	Normal	3	13	2751 ± 796
Tolerant			18	402 ± 94