such a process is active in vivo is not yet known.

These results do not necessarily diminish the significance of the initial observation (*1, 2*) that 5-MTHF participates (at least in vitro) in the metabolism of dopamine inasmuch as the isoquinoline derivatives have been shown to be pharmacologically active and may be of biological significance (*7, 15*). However, speculations about the relation of 5-MTHF–derived psychotomimetic substances and schizophrenia require reevaluation in view of the fact that these specific substances are not the ones being produced.

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Chromatin Fragments Resembling *ν* Bodies

Abstract. *Fragments of chromatin resembling the spheroid chromatin units (ν bodies) have been isolated from formaldehyde-fixed and sonicated chicken erythrocyte nuclei. Ultracentrifugal analyses demonstrated that monomer ν bodies have a molecular weight of about 300,000 per particle, exhibit a protein to DNA ratio (by weight) of 1.22:1, and contain a DNA fragment with a molecular weight of approximately 140,000 per ν body.*

Current models of DNA folding into eukaryotic chromosomes include superhelices (*1*) and particles (*2*) as possible first-level structures in a hierarchy of chromatin packaging. The superhelical model of Pardon and Wilkins (*1*) is based on the occurrence of low-angle x-ray reflections from chromatin fibers (at 110, 55, 38, 27, and 22 Å), their semimeridional orientation, and their susceptibility to stretch. The suggestion that chromatin is composed of particulate subunits (*ν* bodies) is based on the observation that isolated nuclei, swollen in water, fixed with formaldehyde, centrifuged onto electron microscope grids, and positively or negatively stained, revealed linear arrays of spherical particles about 70 Å in diameter connected by thin strands about 15 Å wide (*2*).

We have isolated fractions enriched in monomer *ν* bodies and have measured particle and DNA fragment molecular weights. These studies were performed with isolated chicken erythrocyte nuclei because of the convenience and homogeneity of this material. The fact that *ν* bodies have been observed in several other nuclear systems (*2*) suggests that the results presented here have significance to general models of chromatin organization.

Extensive sonication of water-swollen and formaldehyde-fixed chicken erythrocyte nuclei did not destroy the *ν* bodies but did obliterate the fibrillar arrangement of particles (*2*). The disrupted chromatin containing particles furnished the starting material for fractionation by sucrose gradient ultracentrifugation (Fig. 1A). Electron microscopic analyses of successive fractions from such ultracentrifugation ex-

Table 1. Molecular parameters of monomer *ν* bodies and of *ν*-DNA fragments.

Pools*	*ν* bodies			*ν*-DNA	
	Molecular weight†	$\rho_{CsCl,\ 25°C}$	Ratio of protein to DNA‡	Molecular weight§	$\rho_{CsCl,\ 25°C}$
A	296,000	1.414	1.22	141,000	1.691
B	268,000	1.416	1.19	141,000	1.689
C	330,000	1.413	1.23	142,000	1.690
Average‖ ± S.E.	295,000 ± 13,000	1.414 ± 0.001	1.22 ± 0.01	141,000 ± 2,000	1.690 ± 0.001

* Analyses of positively stained preparations of pools A, B, and C with the electron microscope yielded average monomer particle diameters of 84 ± 6 Å, in reasonable agreement with the range of 60 to 80 Å for monomer *ν* bodies noted previously (*2*). The percentage of monomer particles was also estimated from micrographs for pools A, B, and C to be approximately 64, 50, and 74 percent, respectively. We believe that microscopic estimation of purity of particles tends to minimize the proportion of monomer *ν* bodies, probably due to aggregation of single particles on the electron microscope grid. Sedimentation velocity and sedimentation equilibrium centrifugation analyses of the pools in the guanidine-HCl solvents gave no indications of large proportions of multimers. † Molecular weights of the pools enriched with monomer *ν* bodies were determined by sedimentation equilibrium centrifugation in guanidine-HCl solutions ranging from 2 to 6.5*M*, buffered with 0.02*M* tris (*p*H 7.0). Partial specific volume ($\bar{\nu}$) was calculated from a plot of $(\eta/\eta_0)S$ versus ρ (where η is viscosity; S, sedimentation coefficient; and ρ, density) over the same range of solvent molarities (*11*). The plot was linear over the range of 2 to 6.5*M* guanidine-HCl and yielded a $\bar{\nu}$ of 0.747 ± 0.011 (ml/g), calculated by extrapolation of the value of ρ corresponding to zero sedimentation rate. Data from the sedimentation equilibrium experiments usually yielded linear plots of log concentration versus r^2 (where r is the distance from the center of rotation). In 4.25*M* guanidine-HCl, equilibrium was usually achieved by 86 hours at 6000 rev/min, 20.7°C. Relative viscosities and densities of the guanidine-HCl solutions were calculated from empirical equations (*12*), with molarities and weight fractions of each solution determined by refractometry (*13*). ‡ The ratio of protein to DNA (by weight) was calculated from $\rho_{CsCl,\ 25°C}$ as described by Brutlag *et al.* (*14*), in which we used ρ of chicken DNA as 1.694 (our data) and ρ of histones, 1.245 (*14*). § DNA fragments derived from various pools of *ν* bodies after extensive digestion with Pronase. Samples of pools A, B, and C were dispersed in 0.35*M* of the sodium salt of ethylenediaminetetraacetic acid, 0.7 percent sodium dodecylsulfate (SDS), and 7 m*M* tris (*p*H 9.5) and heated for 30 minutes at 65°C; Pronase was added to a concentration of 500 μg/ml, following a procedure developed by Kavenoff and Zimm (*15*). Digestion was allowed to proceed at 50°C for 6½ hours, and the resulting digest was dialyzed extensively against SSC buffer [0.15*M* NaCl and 0.015*M* sodium citrate (*p*H 7.0)] at room temperature. We measured the molecular weights by sedimentation equilibrium ultracentrifugation in SSC buffer, assuming a $\bar{\nu}_{DNA}$ of 0.503 (*16*). ‖ Averages and standard errors (S.E.) are of all reliable determinations, *not* averages of the average value for each pool. The data presented in this table were calculated on an Olivetti Programma 101 calculator with the use of the programs of Trautman (*17*). The molecular weights calculated from sedimentation equilibrium ultracentrifugation, in which the programs of Trautman were used, represent the weight-average molecular weight at the midpoint of r^2 in the sample solution.

periments revealed that monomer particles were concentrated within the slowly sedimenting side of the single broad peak of ultraviolet-absorbing material. The peak and rapidly sedimenting side of the peak contained increased amounts of contamination by multimers of ν bodies. Based on electron microscopic observations, fractions highly enriched in monomer ν bodies were selected from each centrifuge tube and pooled. Pool A was obtained in this manner (see Table 1). To obtain greater purification of monomer particles, large amounts of material similar to pool A were isolated from several gradients and recentrifuged on 5 to 20 percent sucrose gradients. Fractions were selected as above and combined for pools B and C (see Table 1). Small amounts of purified materials were obtained [that is, each of the pools A, B, and C consisted of 5 to 10 ml at an absorbance at 260 nm ($A_{260\ nm}$) of about 1 to 2].

Molecular weights were measured in a Spinco model E analytical ultracentrifuge equipped with ultraviolet optics, scanner, and multiplexer units. Equilibrium sedimentation analyses of the particle-enriched pools were performed in solvents containing high concentrations of guanidine-HCl (*3*) in order to minimize particle-particle interactions as well as to suppress electrostatic effects (*4*). A summary of the molecular parameters of the pools enriched in monomer ν bodies, and of DNA fragments obtained from these pools by extensive digestion with Pronase, are presented in Table 1. These data indicate that monomer ν bodies appear to have a molecular weight of about 300,000 per particle and to contain a DNA fragment with a molecular weight of about 140,000. Taking the average value of the ratio of protein to DNA (by weight) of ν bodies at 1.22 : 1 and the average measured molecular weight of the DNA (141,000), we calculated a weight per particle of 311,000. The DNA fragment lengths were also estimated by electron microscopic examination by the cytochrome spreading method (*5*). Analysis of histograms of DNA fragment lengths (Fig. 1B) permitted computation of apparent number, weight, and z-average lengths. Pools A, B, and C exhibited number-average lentghs ($\bar{L}_n$) of 803 ± 23, 718 ± 17, and 636 ± 17 Å, respectively. Data from the most homogeneous pool (C) yielded $\bar{L}_z:\bar{L}_w:\bar{L}_n = 1.40:1.17:1$, and a weight average molecular weight of 142,000 ± 3,000 (*6*). Sonicated and unfractionated chromatin was considerably more heterogeneous: $\bar{L}_z:\bar{L}_w:\bar{L}_n = 2.18:1.49:1$. The DNA fragments obtained from the different particle pools appeared to be predominantly double-stranded on the basis of density in cesium chloride, electron microscopy, and thermal denaturation behavior. Using a molecular weight of 670 per nucleotide pair (*6*), one can calculate that a DNA fragment with a molecular weight of 140,000 would contain about 210 nucleotide pairs, with a length of about 710 Å, packed into a spherical particle about 70 Å in diameter (in the dry state).

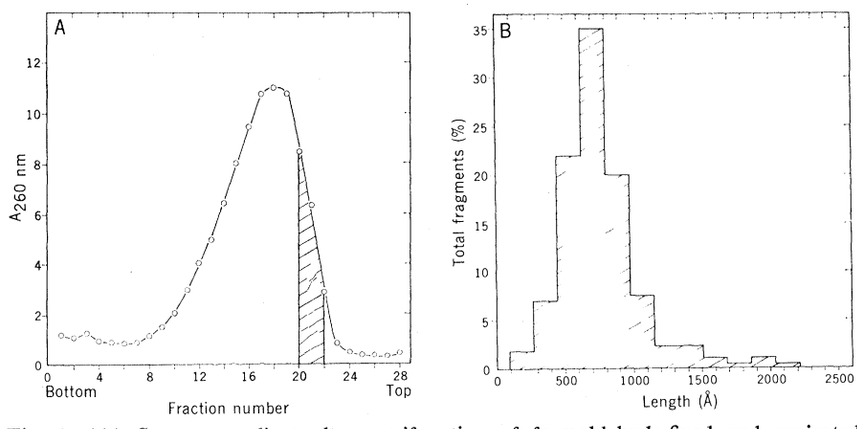

Fig. 1. (A) Sucrose gradient ultracentrifugation of formaldehyde-fixed and sonicated chicken erythrocyte chromatin. Isolated chicken erythrocyte nuclei (*18*) consisting of 100 to 200 mg of DNA were washed and centrifuged twice in 40 ml of cold (4°C) CKM buffer (*19*), once with cold 0.2*M* KCl, and diluted 100-fold with cold distilled water. The nuclei were allowed to swell for 30 to 45 minutes in the cold with intermittent vigorous shaking, then the diluent was made to 1 percent formaldehyde by the addition of fresh 10 percent formaldehyde (*p*H 7.0), and the nuclei were allowed to fix for 18 hours in the cold. The supernatant above the settled nuclei was removed by suction, and the loose nuclear pellet was centrifuged for 15 minutes at 17,000 rev/min in a Sorvall RC2B refrigerated centrifuge. Ten milliliters of the swollen gel pellet, containing 25 to 50 mg of DNA, were placed in a rosette flask cooled by an ice-brine bath and were sonicated with a Biosonik II sonicator (VWR Industries, Rochester, N.Y.) with standard ½-inch (1 inch = 2.54 cm) titanium tip at maximum intensity for 6 minutes (that is, three bursts, 2 minutes each). One-half milliliter of ruptured chromatin was layered over 12 ml of a preformed 5 to 30 percent sucrose gradient (unbuffered in water) in tubes for the Beckman SW-41 Ti rotor. Samples were centrifuged for 17 hours at 41,000 rev/min at about 5°C, tubes were punctured, and fractions were collected by counting drops. The shaded region of the peak indicates the positions of fractions, enriched in monomer ν bodies, which were pooled. Electrophoretic analyses of the chromatin proteins extractable from washed chromatin erythrocyte nuclei by high concentrations of SDS-containing buffers indicated that at least 94 percent of the chromatin protein was histones; less than 6 percent was identifiable as nonhistone proteins. (B) Histogram of DNA fragment lengths from a pool enriched with monomer ν bodies (pool B).

Our observation that DNA fragments from fractions enriched with monomer ν bodies contain about 210 nucleotide pairs is in agreement with the suggestion of Kornberg (*7*). Fragments of unfixed calf thymus chromatin have been prepared by digestion with micrococcal nuclease (*8*). These materials appear to have a particle molecular weight and a DNA fragment molecular weight almost exactly one-half those reported in this study. Although more detailed studies are warranted, it is tempting to suggest that micrococcal nuclease yields chromatin particles that are fragments of ν bodies.

The average molecular weight of protein per particle can be calculated from the difference between particle and DNA fragment weights, or from the ratio of protein to DNA and the DNA fragment weight, and appeared to be 160,000 to 170,000 per ν body. In our previous study (*2*) we pointed out that summing molecular weights for pairs of each of the five major histone classes [that is, F2A1, F3, F2A2, F2B, and F1 (or F2C)] yields a total molecular weight of approximately 151,000. Later, evidence from other laboratories (*7*) indicated that in neutral aqueous solution two of the histones associate into a stoichiometric complex with the molecular composition $(F2A1)_2(F3)_2$. On the basis of these studies, Kornberg (*7*) suggested that pairs of F2A1, F3, F2A2, and F2B histones are associated with

lengths of DNA of approximately 200 nucleotide pairs. Clearly, determination of an exact molecular composition of the histones within ν bodies must await measurements of stoichiometry from isolated chromatin particles.

Our studies (*2, 9*) continue to support the view that the ν bodies are either real structures of native chromatin or represent vestiges of native chromatin periodicities. We employed the low-angle x-ray reflections of chromatin (*1*) as a criterion of the native state and observed that swelling nuclei in low ionic strength solvents and fixing the nuclei with formaldehyde does not destroy these characteristic reflections (*2, 9*). Lyophilization of unfixed chromatin and examination in the dry state does produce perturbations of the x-ray reflections, as described by Pardon and Wilkins (*1*) and by us (*2, 9*). However, rewetting of these chromatin samples results in a return of the x-ray reflections. It is very likely that the dehydrating conditions during electron microscopy lead to specimen shrinkage. [If ν bodies behave as do ribosomes (*10*), the dehydrated volume per particle would be approximately one-half the hydrated volume; that is, 70-Å particles would be derived from hydrated particles about 90 Å in diameter.] Nevertheless, since hydrated formaldehyde-fixed chromatin is sonicated into fragments that resemble monomer ν bodies in the electron microscope, it appears that periodic "weak" points exist in chromatin which do not depend upon dehydration of the material. More definitive verification of the existence of periodic subunits in native chromatin could be obtained by electron microscopic studies of unstained material in a hydration chamber.

Assuming the existence of ν bodies in native chromatin, one could analyze the ways in which a particulate chromatin substructure might relate to the characteristic low-angle x-ray reflections. There are three possible relations that might be envisioned: (i) the x-ray reflections arise solely from interparticle spacings of the packing lattice; (ii) they arise from the structure of the particles (for example, the folding of DNA within each ν body); or (iii) some of the reflections arise from the packing lattice, others from intraparticle structure. Our preliminary observations have shown that pool A exhibits the low-angle reflections; that is, the periodic structure has survived fixation, sonication, and fractionation of the chromatin. A careful study of these low-angle x-ray reflections with changes in concentration of the monomer ν bodies should help one to decide among the suggested models of periodicity within chromatin.

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Coevolutionary Race Continues: Butterfly Larval Adaptation to Plant Trichomes

Abstract. *Plant trichomes can act as effective defenses against herbivores, but at least one species of ithomiid butterfly,* Mechanitis isthmia, *has evolved a unique adaptation for avoiding the trichomes on its spiny* Solanum *hosts. The larvae are gregarious and together they spin a fine silk scaffolding over the tops of the spines on which they can crawl and feed in safety.*

Plants have evolved many different chemicals that act as defenses against herbivore consumption (*1*). Gilbert (*2*) and Levin (*3*) have documented that plant hairs, or trichomes, can act as structural defenses against herbivores. Gilbert (*2*) found that the hooked trichomes on *Passiflora adenopoda* leaves could entrap and fatally wound heliconiine butterfly larvae, thereby acting as an effective defense against larval feeding. Gilbert suggests that the trichomes may be an absolute defense against these larvae and that the coevolutionary race has possibly been won by the plant. We report a previously unknown feeding behavior in a butterfly larva, *Mechanitis isthmia* Bates (Ithomiidae), that is adapted to avoid trichomes. This observation suggests to us that the coevolutionary race has not yet been won by plants with trichomes.

In the American tropics the butterfly family Ithomiidae has radiated extensively on plant species in the Solanaceae (*4*). This adaptive radiation is similar to that of the Heliconiidae on *Passiflora* although far more species are involved. So far as is known ithomiid larvae feed exclusively on solanaceous plants; some species are very specialized feeders while others feed on species in many genera (*4, 5*). The solanaceous plants, in turn, have evolved chemical defenses, including deadly alkaloids (*6*), and many species have evolved trichomes varying from soft pubescence to glandular stinging