tract was then ultrafiltered (4°C) on an Amicon XM-50 membrane [molecular weight (M.W.) cutoff 50,000] and afterward concentrated and dialyzed into physiologic saline with an Amicon UM-2 membrane (M.W. cutoff 2,000) to yield a protein concentration of 5 mg/ml. This cartilage-derived material with a molecular weight between 2,000 and 50,000 is essentially free of uronic acid and hydroxyproline and is enriched in the collagenase inhibitor. After being passed through a Millipore filter (0.22 μm pore size) for sterilization, portions were stored frozen at −70°C until tested. For bone cultures, the cartilage-derived inhibitor was diluted with medium to yield a concentration of 300 μg of protein per milliliter.

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8. Bones from culture were placed in 2.5 percent glutaraldehyde in 0.1*M* cacodylate buffer, *p*H 7.4. After fixation, the undecalcified specimens were rinsed in cacodylate buffer containing 3 percent sucrose and postfixed in 2 percent OsO_4 in 0.2*M* collidine buffer. Specimens were then dehydrated in graded alcohols and embedded in Epon 812 for longitudinal sectioning. Sections 3.0 μm thick were stained with toluidine blue and observed under light microscopy for quantitative histometry. The quantitative data were obtained by examining six midsagittal sections from each bone shaft without knowledge of slide identification, according to the method of D. Rowe and E. Hausmann [*Calcif. Tissue Res.* **20**, 53 (1976)]. Osteoclasts were identified by their large size, multiple nuclei, and characteristic cytoplasmic organellar features. Ultrathin sections were placed on Parlodian-coated 150-mesh copper grids and stained with 5 percent uranyl acetate and 2 percent lead citrate. A Phillips 300 electron microscope was used to examine these prepared specimens.
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Nonhistone Proteins HMG_1 and HMG_2 Change the DNA Helical Structure

Abstract. *Two chromatin nonhistone proteins (from calf thymus) of the high mobility group, HMG_1 and HMG_2, reduce the linking number (topological winding number) of a circular DNA if the covalent closure of the DNA is carried out in their presence. This indicates that these proteins can either unwind the double helix, or induce a supercoiling of the DNA.*

Extraction of calf thymus chromatin with 0.35*M* NaCl yields two groups of nonhistone proteins. On the basis of their electrophoretic mobilities on gel, the two groups have been referred to as the low mobility group and the high mobility group (HMG) (*1*). The HMG group consists of four proteins designated HMG_1, HMG_2, HMG_{14}, and HMG_{17}. All four proteins have been purified to homogeneity, and the amino acid sequence of one of them (HMG_{17}) has been determined (*2*).

Physicochemical studies of HMG_1, HMG_2, and HMG_{17} have been undertaken (*3*, *4*). HMG_1 and HMG_2 are similar to each other, each containing about equal molar amounts of acidic and basic amino acids (*1*). Their molecular weights are approximately 26,000 (*5*, *6*), and both have globular structures with approximately 45 percent of the residues in the α-helix configuration (*7*, *8*). The two proteins interact with DNA nonspecifically and form soluble complexes (*5*). The proteins destabilize the DNA double helix, as indicated by a lowering of the melting temperature of DNA in the presence of the proteins (*3*).

We now report that both HMG_1 and HMG_2 proteins alter the configuration of the DNA double helix. Circular PM2 DNA containing one single-chain scission per molecule was converted to the covalently closed form by *Escherichia coli* DNA ligase in the presence of increasing amounts of either HMG_1 or HMG_2. On removal of the bound protein, the electrophoretic mobility of the DNA in agarose gel was examined. The electrophoretic pattern of the control DNA sample ligated at 24°C in the absence of HMG_1 and HMG_2 is shown in Fig. 1a. The electrophoresis condition was such that the group of covalently closed DNA bands were all negatively supercoiled. This can be achieved by carrying out electrophoresis at 4°C in an Mg^{2+}-containing buffer (*9*). As was interpreted previously, a group of covalently closed DNA bands differing in linking numbers (topological winding numbers) are formed because of thermal fluctuation of the DNA helix configuration (*10*). When increasing amounts of HMG_1 were present in samples during treatment with ligase, the covalently closed DNA formed showed a progressive increase in electrophoretic mobility (Fig. 1, b to g). This increase in mobility results from an

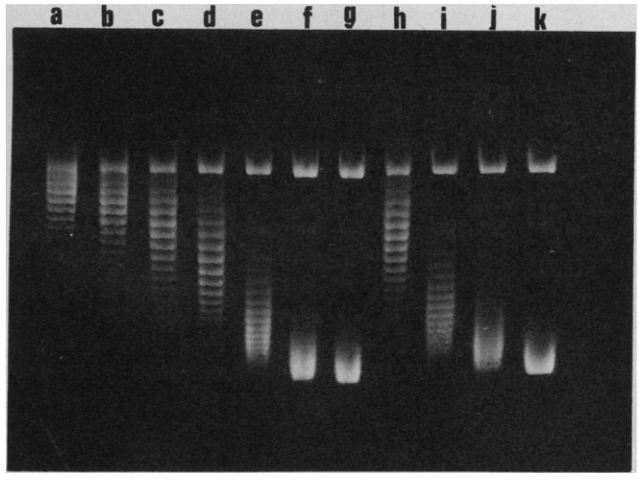

Fig. 1. Reaction mixtures (86 μl each) contained 18 m*M* tris-HCl, *p*H 8.0, 2.7 m*M* $MgCl_2$, 2.3 m*M* potassium phosphate, 44 μg of bovine plasma albumin per milliliter, 29 μg of NAD per milliliter, 0.9 m*M* EDTA, 0.5 m*M* dithiothreitol, 6 percent (by volume) glycerol, 13 μg of PM2 DNA (containing one single-chain scission per molecule) per milliliter, and varying amounts of HMG_1 and HMG_2 proteins. The reaction mixtures were incubated at 24°C for 10 minutes, and 4 μl of an *E. coli* ligase stock (in the same reaction medium) was added to each tube, and incubation was continued for another hour. The reaction was stopped by the addition of 15 μl of solution containing 25 percent (by volume) glycerol, 5 percent sodium dodecyl sulfate, and 0.25 mg of bromophenol blue per milliliter. Half of the volume of each stopped reaction mixture was placed on an agarose (0.7 percent) slab gel for electrophoresis in buffer containing 5 m*M* magnesium acetate, 40 m*M* tris-HCl, *p*H 8.0, 1 m*M* EDTA (trisodium salt). Electrophoresis was carried out at 4°C and at 3 V/cm for 39 hours. The numbers of HMG_1 molecules per DNA molecule during the ligase treatment were: (a) 0, (b) 40, (c) 80, (d) 120, (e) 160, (f) 240, and (g) 320. The numbers of HMG_2 molecules per DNA molecule were (h) 50, (i) 100, (j) 150, and (k) 200. The HMG proteins were prepared by the method of Johns *et al.* (*1*). Concentrations of HMG_1 and HMG_2 were determined spectrophotometrically taking $E^{280}_{1\%} = 82$. A value of 6.5×10^6 was used for the molecular weight of PM2 DNA.

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increase in the degree of negative superhelicity. In other words, DNA covalently closed in the presence of HMG_1 has a lower linking number than DNA covalently closed in the absence of this protein. Similar results were obtained with HMG_2 (Fig. 1, h and k).

The results were quantified as described for the unwinding of the DNA helix by *E. coli* RNA polymerase (*11*). For HMG_1, the average linking number of PM2 DNA molecule in samples covalently closed in the presence of 40, 80, 120, and 160 protein molecules per DNA molecule were reduced by 1.2, 2, 3.4, and 6.8 turns, respectively. For HMG_2, samples covalently closed in the presence of 50 and 100 protein molecules per DNA were found to have 3.2 and 8.5 turns, respectively. On the basis of the measurements of Shooter *et al.* (*5*), we believe that most of the protein molecules present during the ligase treatment were bound to the DNA.

A reduction in the linking number of the DNA by the presence of a protein during the covalent closure of the DNA could arise by a number of mechanisms. These include supercoiling of the DNA around a protein core, separation of DNA strands by cooperative or noncooperative binding of protein molecules to single-stranded DNA segments, and a change in DNA helix rotation by the binding of protein molecules. The lowering of the melting temperature of DNA by these proteins (*3*) suggests that the proteins probably uncoil the DNA double helix. How HMG_1 and HMG_2 affect the DNA structure remains to be determined.

HMG proteins are present in various tissues of a number of animals (*1*, *12*) and they have been found in nucleosomes (*13*). On the basis of the yield of these proteins, it has been estimated that the molar ratio of HMG_1 and HMG_2 to total histones is about 1 : 50. In other words, there is on the average approximately one molecule of either HMG_1 or HMG_2 per ten nucleosomes. Our observation that HMG_1 and HMG_2 can affect the helical twist or the tertiary coiling of the DNA points to the possibility that these proteins might play a structural role in the higher order coiling of the nucleosomes. The possibility also exists that by modifying the structure of the DNA helix, these proteins could affect processes such as transcription.

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Isolation of St. Louis Encephalitis Virus from Overwintering *Culex pipiens* Mosquitoes

Abstract. *Two strains of St. Louis encephalitis virus were isolated from overwintering mosquitoes collected in Maryland and Pennsylvania during January and February 1977. These isolations from* Culex pipiens *constitute evidence that a mosquito-borne flavivirus can persist in a vector mosquito in temperate climates during the winter season.*

St. Louis encephalitis (SLE) virus leads all other arboviruses in causing human disease in the United States, and numerous epidemics have occurred since its initial detection in 1933. The virus is maintained in nature during the summer and fall by a mosquito-avian-mosquito cycle. At least three mosquito species, *Culex pipiens*, *Culex nigripalpus*, and *Culex tarsalis* have been incriminated as vectors. The seasonal distribution of human disease coincides with high infection rates in mosquitoes and birds during late summer and early fall, and human infections tend to be more prevalent in urban areas, especially in the eastern United States where the domestic mosquito *C. pipiens* is the principal vector.

Although details of the summer and fall mosquito-avian-mosquito cycle are relatively well known, the mechanism by which the virus persists during the winter season is obscure. Reeves (*1*) reviewed several hypotheses to explain the survival of arboviruses through these adverse periods, including one that hibernating vector mosquitoes served as overwintering hosts. In temperate regions, *C. pipiens* and *C. tarsalis* overwinter solely as inseminated females. The fact that these *Culex* mosquitoes are the primary vectors of SLE virus during the summer and fall has prompted several investigators to test the overwintering hypothesis. The SLE virus has been shown experimentally to persist for more than a month in *Culex* species (*2*, *3*). A single isolation of SLE virus was obtained from *C. tarsalis* females collected during March in the western United States (*4*). Other isolations of arboviruses during the winter include one strain of western equine encephalitis virus from *C. tarsalis* in Colorado (*5*) and two Japanese encephalitis virus strains from *C. pipiens* in Korea (*6*).

In spite of these findings, the hypothesis that hibernating mosquitoes harbor arboviruses over the winter has not gained wide acceptance. This is partly because of the belief that blood feeding in *Culex* mosquitoes is drastically reduced or suspended shortly before they hibernate so that their chance of taking a viremic blood meal before overwintering is exceedingly small or nonexistent (*2*). Furthermore, other investigations (*7*) have shown that parous females are seldom found among collections of overwintering mosquitoes, and those which are found are usually observed in early winter and are presumed not to survive until spring. However, ovarian diapause in laboratory-reared *C. pipiens* has been demonstrated (*8*). In these cases, females, experimentally induced to hibernate, took full blood meals but, in most instances, ovarian development did not follow. If this situation occurred in nature, such females would prove to be nulliparous upon dissection, in spite of having taken a blood meal. Nulliparity, then, would not be proof of the failure of the mosquito to obtain a blood meal shortly before hibernating.

Since the 1975 SLE epidemic, we have conducted investigations to reexamine

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