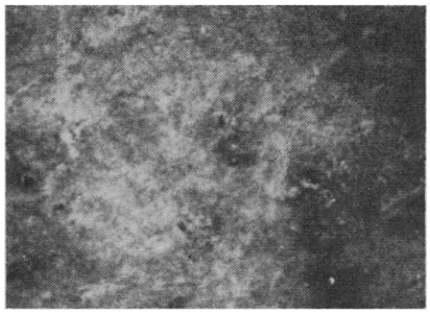

Fig. 2. Normal human cerebral white matter stained with fluorescein-conjugated goat antiserum to human 7*S* γ-globulin alone. No specific fluorescence noted. (About × 300)

the antiserum to 7*S* γ-globulin during the preparation of the conjugated antisera. Heating the serum to 56°C for 30 minutes did not alter the staining pattern suggesting that this reaction is not dependent upon complement as was shown by Bornstein (*2*).

Concentration of cerebrospinal fluid of 5 to 100 times resulted in a pattern of fluorescence not significantly different from that of serum except that it was less intense, and fewer glial cells and myelin sheaths were stained. Such fluorescence was seemingly solely dependent on the concentration of γ-globulin in the cerebrospinal fluid and was not observed with samples of whole cerebrospinal fluid except in two instances when the γ-globulin concentration was greater than 4 mg/ml. Concentrated samples produced detectable fluorescence at approximately 0.8 mg/ml which preliminary studies had suggested as the approximate concentration needed to demonstrate fluorescence with diluted normal serum.

The fluorescence of glial cells and myelin sheaths produced by both serum and cerebrospinal fluid was markedly diminished by the application of noncein-conjugated antisera to 7*S* γ-globubefore staining with the conjugated antiserum. The application of fluorescein-conjugated antisera to 7*S* γ-globulin and the other globulins alone elicited no fluorescence of glial cells or myelin sheaths and elicited only mild fluorescence of blood vessel walls, pial connective tissue, and neuronal nuclei (see Fig. 2).

Except for the tissue from the two patients with amyotrophic lateral sclerosis all human tissues tested reacted in the same manner. In these two instances the region of the lateral funiculus of the spinal cord, representing areas of pyramidal tract involvement, revealed rare, pale myelin sheaths surrounded by a dense intensely fluorescing network of glial fibers. Similar fluorescence of glial fibers was observed after application of the conjugated antisera alone. The significance of this observation in these two cases is not apparent. The reactions of human serum and cerebrospinal fluid with monkey tissue were similar to those described, as were the reactions between monkey serum and the homologous monkey tissue, even though fluorescein-conjugated antimonkey globulin was not used.

The fluorescence of glial cells and myelin sheaths produced consistently by all the serum and cerebrospinal fluid samples tested suggests that this is not an autoantibody in the strict sense. There appears to be, as yet, no significant qualitative difference between the γ-globulins from either cerebrospinal fluid or serum or among the various conditions as studied by this technique. This absence of discernible differences in the staining pattern raises the question of nonspecific protein interaction. Such interactions have been shown to result from electrostatic forces between the fresh frozen tissue and the serum proteins (*9*) or may be related to the inherent pinocytotic properties of glial cells during life (*10*) as has been shown for granulocytes and histiocytes (*9*).

Our studies suggest that the affinity of 7*S* γ-globulin for the glial cells of white matter and for myelin sheaths may represent more than a nonspecific interaction. Such specificity was not seen with the other major protein fractions of serum and cerebrospinal fluid or with the fluorescein-conjugated antisera alone. This affinity may in some way be related to glial cell metabolic functions in normal, and perhaps, pathological states or may simply represent cross relationships between existing antibodies.

The concomitant fluorescence of myelin sheaths with glial cells is of note in view of the concept that the sheath is derived from modified glialcells and myelin sheaths may be peof 7*S* γ-globulin for primate glial cells and myelin sheaths may be peculiar to this highest animal order since this phenomenon has not been described in such experimental animals as the rat, mouse, guinea pig, or rabbit. Such an affinity must be explained for the normal state in order to assess properly the presence or significance of autoantibodies in the human demyelinating diseases and experimental "allergic" encephalomyelitis.

C. DOMINIQUE ALLERAND
MELVIN D. YAHR
Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York

References and Notes

1. E. H. Beutner, E. Witebsky, N. R. Rose, J. R. Gerbasi, *Proc. Soc. Exptl. Biol. Med.* **97**, 712 (1958); A. L. Sherwin, M. Richter, J. B. Cosgrove, B. Rose, *Science* **134**, 1370 (1961); E. J. Field, A. Ridley, E. A. Caspary, *Brit. J. Exptl. Pathol.* **44**, 631 (1963).
2. M. B. Bornstein, *Natl. Cancer Inst. Monograph* **11**, 197 (1963).
3. M. D. Yahr, S. S. Goldensohn, E. A. Kabat, *Ann. N.Y. Acad. Sci.* **58**, 613 (1954).
4. W. B. Chodirker and T. B. Tomasi, Jr., *Science* **142**, 1080 (1963).
5. C. F. C. MacPherson and J. B. R. Cosgrove, *Can. J. Biochem. Physiol.* **39**, 1567 (1961).
6. E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., ed. 2, 1961), pp. 366–369.
7. Microbiological Associates, Bethesda, Md.; The Sylvana Co., Millburn, N.J. The duck antihuman 7*S* γ-globulin was prepared by Dr. A. J. L. Strauss.
8. A. N. Rahman and C. N. Luttrell, *Bull. Johns Hopkins Hosp.* **110**, 6 (1962).
9. R. C. Nairn, Ed., *Fluorescent Protein Tracing* (Livingstone, Edinburgh, 1962), pp. 116–121.
10. I. Klatzo and J. Miquel, *J. Neuropathol. Exptl. Neurol.* **19**, 475 (1960).
11. R. P. Bunge, M. B. Bunge, H. Ris, *J. Biophys. Biochem. Cytol.* **10**, 67 (1961).
12. We thank K. Hsu and B. Seegal of the Department of Microbiology and J. Zabriskie of the Rockefeller Institute for invaluable technical assistance. Work supported by NIH grants NB-04891-01 and B-3359. One of us (C.D.A.) is a fellow, National Multiple Sclerosis Society.

31 March 1964

DNA from Bacteriophage Lambda: Molecular Length and Conformation

Abstract. *The length of the DNA molecules from wild-type λ bacteriophage is 17.2 μ, corresponding to a molecular weight of 33 million in the B form. The molecules from a double-deletion mutant of λ are 23 percent shorter. Both types of molecules join ends at 60°C to form circular molecules or polymers. The point of junction cannot be distinguished by any irregularity in the uniform duplex molecule.*

We have measured the lengths of unbroken DNA molecules from lambda bacteriophage as seen in electron micrographs, calculated their true length, and related this length with the molecular weight of DNA from the same bacteriophage as judged by other kinds of

experiments. There are two principal errors that can affect these results: the distortion of the lengths of the molecules when immobilized on the microscope grids and the variability in the magnification. In order to check for possible stretching or shrinking, grids were prepared from the same solution of λ DNA by two quite different procedures—the streaking procedure of Beer (*1*) whereby the molecules are deposited on a smooth carbon film during flow, and the Kleinschmidt procedure (*2*) whereby the molecules are floated on a clean water surface after combining with cytochrome *c*, a basic protein. Micrographs of a grating replica were interspersed to assess the magnification of the electron microscope and its variability. To compare molecules of different sizes, the two different types of grids were prepared from solutions of DNA of wild type λ and the density mutant λb_2b_5. The DNA from λb_2b_5 should be shorter, as a result of two deletion mutations (*3*). Finally, both wild and mutant DNA's were subjected to the conditions reported by Hershey *et al.* (*4*) to produce a faster-sedimenting form of the λ DNA molecule which they termed "folded." Grids prepared from these solutions showed a large proportion of circular molecules.

Lysates of "wild" (λ$cb_2^+b_5^+$) and "mutant" (λcb_2b_5) phage grown in a peptone-tris-broth (*6*) culture of *Escherichia coli* W3110 were filtered through celite and an HA Millipore filter. The phage was concentrated from the filtrate by differential centrifugation and the DNA obtained by extracting the phage twice with phenol by gently rolling in a closed tube at 60 rev/min for 30 minutes. After removal of the phenol by dialysis, the DNA was purified by chromatography on hydroxyapatite (*7*) and diluted to 5 μg/ml in 0.60*M* NaCl, 0.05*M* phosphate buffer, *p*H 6.8. This solution was heated to 75°C for 10 minutes and either quickly cooled to promote the formation of linear monomers, or incubated at 60°C for 30 minutes to cause the formation of ring-shaped molecules (*4*). Carbon grids were prepared as before (*8*). Grids with cytochrome *c* were prepared by spreading 0.08 ml of a solution, containing 0.7 μg/ml DNA in 2.2*M* NaCl and 0.02 percent cytochrome *c* (*9*), onto a 60-cm² surface of double-distilled water with a flame-cleaned stainless-steel blade. The invisible film

Table 1. Frequency of various molecular types after treatment. Two solutions of DNA from wild type λ bacteriophage (5 μg/ml in 0.60*M* NaCl) were heated to 75°C for 10 minutes. One was quickly cooled and the other was slowly cooled, and then each sample was examined by the cytochrome *c* film procedure. Both linear and circular monomers had lengths of 17.4 ± 2 μ, while the lengths of the dimers were between 30 and 40 μ. "Flowers" were multiple-looped structures which may be assumed to have resulted from intramolecular cross-linking in the cytochrome solution. The "unmeasurable" molecules were so tangled that visual separation of individual strands was impossible. The "fragments" had lengths less than 12 μ and were presumed to be breakage products.

Monomers		Dimers		Flowers	Unmeasurable	Fragments
Linear	Circular	Linear	Circular			
			Quickly cooled			
45	0	0	0	13	10	17
			Slowly cooled			
19	25	6	1	52	17	3

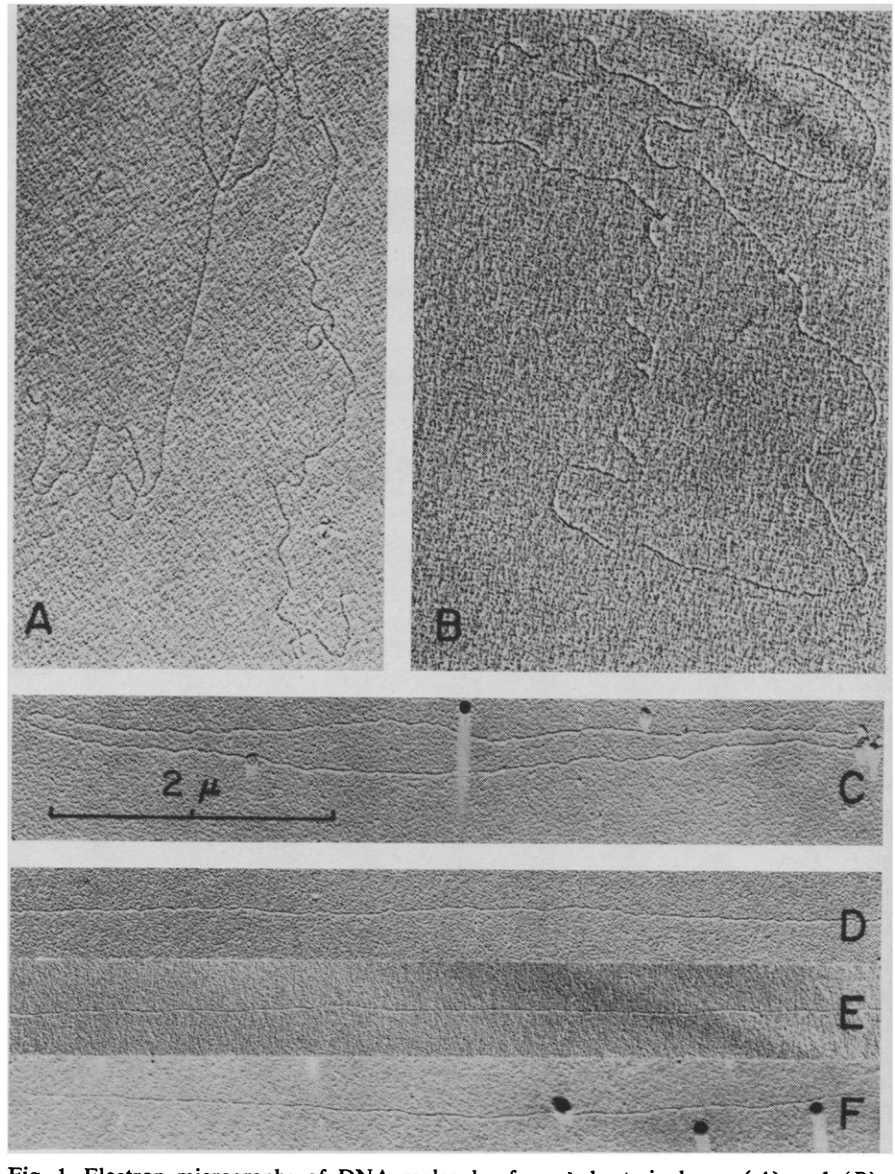

Fig. 1. Electron micrographs of DNA molecules from λ bacteriophage. (*A*) and (*B*) Linear and circular forms, cytochrome *c* preparation of λ$cb_2^+b_5^+$ DNA which had been heated to 60°C for 30 minutes. (*C*) Circular form, streaked-on-carbon preparation of λcb_2b_5 DNA which had been heated to 60°C for 30 minutes. (*D*) (*E*) and (*F*) Middle regions of dimers, carbon preparation of λ$cb_2^+b_5^+$ DNA which had been heated to 60°C for 30 minutes. The chances are very good that the "joint" between the two molecules is located in each of these pictures. However, no unique feature is visible in them. Magnification of all six micrographs is about × 19,000.

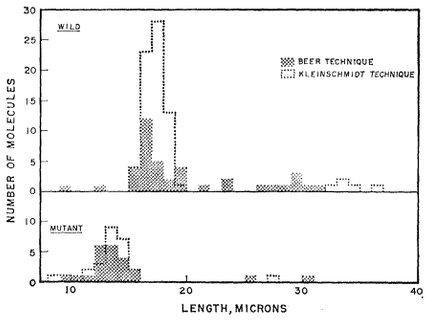

Fig. 2. Histogram showing molecular lengths. (Top) A single batch of DNA molecules from $\lambda cb_2^+b_5^+$, containing both linear and circular forms, measured after electron microscopic preparation by the two different techniques. Both yield the same value for monomer length (both linear and circular), while the more stressful technique of Beer results in more scattered lengths being seen. Dimers occur in both preparations. (Bottom) DNA molecules from the density mutant λcb_2b_5. Those prepared by the technique of Beer had been heat-treated to favor the formation of circles, while those prepared by the technique of Kleinschmidt had received no heat treatment.

was touched with a grid covered by a carbon film, dried, and shadowed with platinum at an angle of 7° in two or three different directions. Some results typical of over 260 molecules that were measured are shown in Fig. 1.

The lengths of these molecules were measured by tracing enlarged projections with ink and determining their contour length with a map-distance measuring device. The total magnification was determined by micrographs of a grating replica (*10*). The calculated molecular lengths of 163 molecules are assembled in histograms shown in Fig. 2. Two things can be seen. (i) The wild-type molecules are 17.2 ± 0.9 μ long (96 measurements) while the mutant molecules are 13.2 ± 1.5 μ long (48 measurements) or 23 percent shorter. This is in agreement with other estimates of the fraction of DNA missing from the mutant molecules, namely: 23 percent, calculated from the densities of the phage particles (*3*) and 20 percent, calculated from the relative sedimentation rates of the DNA molecules in sucrose gradients (*6*). (ii) The mean length measured on carbon grids, 17.1 ± 1.1 μ, is the same as that measured in cytochrome *c* films, 17.2 ± 0.8 μ. Since it is unlikely that both procedures would lead to exactly the same perturbation in length, we shall assume that neither has been significantly disturbed. At the time of deposition, the molecules were in solution and thus in their most hydrated form. If this were equivalent to the B crystallographic form (*11*), the sodium salt of the molecule should have a linear density of 192 daltons per angstrom. Multiplying this number by the measured length gives a molecular weight of 33 million for the DNA molecule of wild-type λ. This value for the molecular weight is in good agreement with the value of 31 million obtained by Burgi and Hershey (*12*) from the sedimentation rate of this DNA in sucrose gradients. This agreement encourages the belief that our assumption of the B configuration was justified.

About half of the molecules whose lengths are shown in Fig. 2 were in the circular form. The lengths of circular and linear monomers were the same within the limits of experimental error. Thus the point of union must be at or near the ends of the molecule. To examine this point of union more carefully, we have inspected the central regions of eight linear dimers. Three of these are shown in Fig. 1 (*D*, *E*, and *F*). We can see no obvious joining point.

Finally, we turn to the frequencies of different types of molecules seen in grids prepared from DNA solutions which had received the two different heat treatments. Samples were prepared with cytochrome film and every molecule seen was photographed. Table 1 shows the composition of the total group comprising 97 molecules and fragments. The results leave very little doubt that slow cooling from 75°C or incubation at 60°C does cause the DNA molecule from λ bacteriophage to assume a circular form.

L. A. MacHattie
C. A. Thomas, Jr.
Biophysics Department, Johns Hopkins University, Baltimore, Maryland

References and Notes

1. M. Beer, *J. Mol. Biol.* **3**, 263 (1961).
2. A. Kleinschmidt, D. Lang, R. K. Zahn, *Z. Naturforsch.* **16**, 730 (1960).
3. G. M. Kellenberger, M. L. Zichichi, J. Weigle, *Nature* **187**, 161 (1960); *Proc. Natl. Acad. Sci. U.S.* **47**, 869 (1961); *J. Mol. Biol.* **3**, 399 (1961).
4. A. D. Hershey, E. Burgi, L. Ingraham, *Proc. Natl. Acad. Sci. U.S.* **49**, 748 (1963).
5. Obtained from Dr. Hershey, who obtained them from Dr. G. Kellenberger. We find that the density of λcb_2b_5 phage is 0.022 g/cm^3 less than that of $\lambda cb_2^+b_5^+$ in CsCl. This indicates that the mutant is missing 22 percent of its DNA in agreement with *3*.
6. E. Burgi, *Proc. Natl. Acad. Sci. U.S.* **49**, 151 (1963).
7. Y. Miyazawa and C. A. Thomas, Jr., in preparation.
8. L. A. MacHattie, G. Bernardi, C. A. Thomas, Jr., *Science* **141**, 59 (1963).
9. Type III, Sigma Chemical Co.
10. E. F. Fullam Co. 2160 lines/mm, carbon replica.
11. R. Langridge *et al.*, *J. Mol. Biol.* **2**, 19 (1960).
12. E. Burgi and A. D. Hershey, *Biophysical J.* **3**, 309 (1963).
13. We thank Barbara Chandler who showed us some excellent pictures of circular λ DNA molecules and outlined for us her use of the cytochrome *c* procedure. Work supported by AEC contract AT(30–1)–2119 and by NIH (E-3233). We are indebted to the Damon Runyon Foundation for a fellowship (No. 327) to L.A.M. during this period.

10 February 1964

Density Gradient Centrifugation of a Murine Leukemia Virus

Abstract. *The Rauscher leukemia virus separated as a single band upon density gradient centrifugation in cesium chloride, rubidium chloride, sucrose, potassium citrate, or potassium tartrate. Prolonged exposure to concentrated potassium citrate or potassium tartrate solutions caused lysis of the virus; the resulting products, having different densities, separated on the density gradients.*

Over a dozen viruses (*1*) have been isolated and identified as causative agents of murine leukemias. Several of these have been prepared in large amounts by a procedure involving differential centrifugation (*2*). Electron microscopy (*3*) of the murine leukemia viruses indicates that they are typical "membrane viruses," because, like myxoviruses and viruses of the avian leukosis group, they are assembled at the plasma membrane of the infected cell, from which they are released by a budding process. The virus particles measure approximately 100 mμ and contain denser-staining cores (presumably nucleoprotein) surrounded by lesser-staining double outer membranes (presumably derived from the plasma membrane).

Myxoviruses can be lysed by chemical treatment; the internal structures of various members of this group are serologically interrelated and contain nucleoprotein filaments which show individual differences in morphological fine structure (*4*). Similar filaments have been reported as internal constituents of the avian myeloblastosis virus (*5*) and of virus-like particles associated with two cases of human leukemia (*6*).