Table 1. Serotonin content of the pineal organ (nanograms per gland) at different times in the photoperiod in male Sprague-Dawley rats 8 to 9 weeks old. The experimental rats were ganglionectomized; the controls were sham-operated or intact.

Series	Control rats		Experimental rats	
	Light*	Dark†	Light*	Dark†
I	88	48		
II	117	42	67	53
	97	52	72	70
III	96	33	46	54
IV	134	22	48	
		76		
		76		
V	104	20	51	44
	99		44	84
			40	
VI	112	41	40	41
		Means		
	106	42	53	58
		Range		
	134–88	76–20	72–40	84–41

* Animals killed approximately 8 hours after the onset of the light period. † Animals killed approximately 4 hours after the onset of the dark period.

pathway is important in pineal metabolism. After the removal of the superior cervical ganglia the amount of serotonin in the pineal organ is reduced as measured by spectrofluorometric assays, or as seen in a study of fluorescent sections (*2*). Furthermore, an enzyme which is essential in the transformation of serotonin to melatonin, hydroxyindole-*O*-methyl transferase does not vary in response to continuous light or darkness in ganglionectomized rats (*3*) as it does in intact animals. An interesting aspect of pineal physiology has been demonstrated by Quay (*4*) who has shown that the amount of serotonin in the pineal organ fluctuates diurnally in rats in relation to the photoperiod, the largest amount being found during the 8th hour of the light period and minimum amounts being detected 4 hours after the onset of the dark period. The study reported here was designed to determine whether or not superior cervical ganglionectomy would affect this diurnal rise and fall of serotonin in the pineal organ.

Fifty-eight Sprague-Dawley male rats were placed under controlled environmental conditions when 22 days of age. Throughout the experimental period all animals were exposed to 14 hours of light followed by 10 hours of darkness. The temperature was maintained at 24° ± 1°C, and the humidity at 50 percent. Purina Chow and tap water were available all the time.

Of these animals, 26 were subjected to superior cervical ganglionectomy under ether anesthesia while 30 served as sham-operated or unoperated controls. All operations were performed when the rats were 24 to 28 days old. The completeness of this procedure was evident in the ptosis of the eyelids which developed. This was marked immediately after the operation but with time was partly overcome as the voluntary nerves corrected for the tonus normally maintained by the sympathetic nerve supply. Five to six weeks later the animals were killed quickly by cervical dislocation at a given point in the photoperiod, that is, within 7½ to 8½ hours after the onset of the light phase or 3½ to 4½ hours after the beginning of the dark period. During the dark period the animals were killed in almost total darkness, a substage lamp equipped with a green filter serving as the light source.

Each pineal organ was removed within 1 minute after death and immediately homogenized in a 5-ml centrifuge tube as described by Quay (*4, 5*). To insure that adequate amounts of serotonin were present for accurate measurement, two pineal organs were combined in each tube. All pools were assayed within 24 hours and prior to assay they were maintained at −5°C. The extraction procedure used has been fully described elsewhere (*5*). While this method is not entirely specific for serotonin it does insure the nearly complete removal of interfering indoles known to occur in the pineal body. The fluorescence was measured with a Farrand spectrofluorometer equipped with a 0.3-ml quartz cuvette and adaptor. The preparation of the pools and the extraction procedure were repeated on six different occasions.

As shown in Table 1, pineal organs removed from intact or sham-operated rats approximately 8 hours after the onset of the light period always contained large amounts of serotonin; pineal organs from control rats, removed about 4 hours after the beginning of the dark period, gave significantly lower serotonin values. After the removal of the superior cervical ganglia, there was no such variation in serotonin content in relation to the photoperiod.

These results confirm Quay's observation that the amount of serotonin in the pineal organ of the rat varies with the photoperiod. In addition they indicate that such diurnal fluctuations do not occur if the sympathetic pathways from the superior cervical ganglia to the pineal organ are interrupted.

VIRGINIA M. FISKE
Department of Zoology and Physiology, Wellesley College, Wellesley 81, Massachusetts

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Electron Microscopy of Single-Stranded DNA: Circularity of DNA of Bacteriophage ϕX174

Abstract. *The single-stranded DNA of coliphage ϕX174 has been examined with the electron microscope by a modification of the protein-monolayer-adsorption technique. The molecules were found to be circular with a total length of 1.77 ± 0.13 microns.*

The DNA of coliphage ϕX174 is single-stranded (*1*) and also circular (*2*). The latter conclusion, which has been based upon the resistance of the DNA to digestion by exonucleases and upon an analysis of the change in the ultracentrifugal pattern after the introduction of chain scissions by deoxyribonuclease, is confirmed by electron microscopy.

Direct observation of double-stranded DNA molecules by electron microscopy is easily performed if the DNA is adsorbed onto protein monolayers (*3*). However, making this observation has been complicated by the entanglement of a variable fraction of the molecules, although it is usually possible to find a sufficient number of extended, untangled filaments to obtain a length distribution.

When ϕX174 viral DNA was examined, exceedingly severe tangling eliminated any possible conclusion concerning length or configuration, although the success with the double-stranded replicative form (*4*), that is, the clear

demonstration of ring molecules (*5*, *6*), made it seem likely that the single-stranded form is also circular.

We have assumed that intramolecular hydrogen bonding is principally responsible for entangling and have therefore modified the preparative procedure to minimize such bonding. This modification consists of denaturing the DNA by one of the methods of Freifelder and Davison (*7*, *8*) in order to break all hydrogen bonds and of subsequently handling the DNA in the presence of formaldehyde at concentrations sufficient to prevent re-formation of hydrogen bonds.

The DNA of ϕX174 was prepared according to the method of Sinsheimer (*1*) and adjusted to a concentration of 70 μg/ml in a solution of 0.1*M* NaCl and 0.05*M* tris (*p*H 7.7); this served as a stock. The solution for spreading monofilms was made by mixing the following components in sequence for the indicated times: 0.04 ml DNA + 0.04 ml 1*M* NaOH, 20 seconds; 0.4 ml 37 percent formaldehyde (adjusted to *p*H 11 with 1*N* NaOH), 30 seconds; 0.1 ml 1*M* KH_2PO_4; 0.6 ml 0.01*M* PO_4–0.005*M* EDTA (*p*H 7.8); and 0.4 ml 4*M* NaCl.

One-tenth milliliter of the denatured DNA solution was diluted to contain 0.5 μg of DNA per milliliter by adding 1*M* ammonium acetate containing 0.5 percent formaldehyde (neutralized and boiled for 10 minutes). Finally, 0.1 ml of 0.01 percent cytochrome *c* in 1*M* ammonium acetate was added. This solution was spread, 4 minutes later, by the standard method (*3*) except that the spreading was done on 0.5 percent formaldehyde (pretreated as described) instead of water. Lower concentrations of formaldehyde were not satisfactory. The viscoelastic properties and the spreading speed of the film on this solvent are comparable with the properties and speed obtained with spreading the film on water. The film was transferred to carbonized support films on platinum grids by touching the surface and the adhering droplet was removed by touching an ethanol surface (*6*). After drying with filter paper the preparation was rotary shadowed with uranium at an angle of 6 to 10 degrees. Undenatured ϕX174 DNA was also examined by the standard technique (without treatment with alkali and formaldehyde).

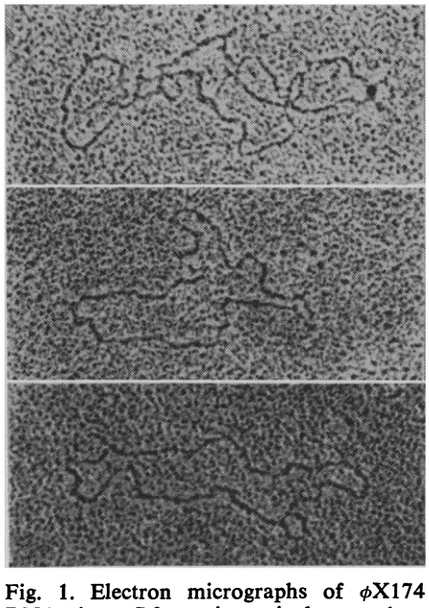

Fig. 1. Electron micrographs of ϕX174 DNA rings; 7.3 cm is equivalent to 1 μ. Total magnification, 7300.

Figure 1 shows representative single-stranded rings of ϕX174 DNA. In a typical field about one-half of the DNA is easily seen as circular molecules; filaments account for fewer than 5 percent. The remainder of the DNA consists of molecules overlapping, aggregated, or tangled, the number of which can be substantially reduced by spreading the DNA at lower concentrations. When they are mixed with other material which is double-stranded (*9*) the single strands appear thinner, are more difficult to see because of lower contrast with the background and, in general, show sharp kinks, an observation consistent with the expected greater flexibility of the single strands. Undenatured preparations (untreated with alkali and formaldehyde) show badly tangled masses, without any visible free ends.

The lengths of most of the rings were measured by tracing a projected enlarged image on paper and placing 0.08-cm diameter polyethylene tubing along the path (*6*). A histogram of the measured lengths of 186 rings is shown in Fig. 2. The mean contour length is 1.77 ± 0.13 μ (7.3 percent), and it is greater than that found for the replicative form (1.7 μ ± 7 percent) (*5*). The greater mean length probably reflects the greater flexibility and less degree of coiling of single-stranded DNA, although some effect of formaldehyde is certainly possible. If the molecular weight is 1.7×10^6 (*1*) the mass per unit length of molecules prepared in this way is 0.95×10^6 dalton/micron.

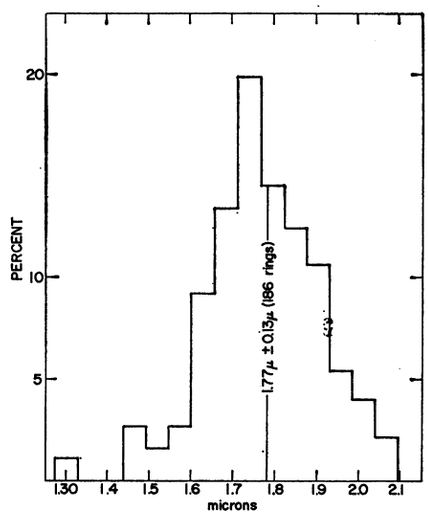

Fig. 2. Histogram of length distribution of 186 ϕX174 DNA rings. The error shown for the mean is the standard deviation.

The small number of filaments is an indication of the safety of the alkali treatment for denaturating DNA (*7*) and furthermore reflects the stability of the linkage that causes ring closure. We assume that this linkage is probably a covalent bond. This is consistent with the resistance of infectious ϕX174 DNA to alkali (*10*).

DAVID FREIFELDER
Donner Laboratory, University of California, Berkeley

ALBRECHT K. KLEINSCHMIDT
Virus Laboratory, University of California, Berkeley

ROBERT L. SINSHEIMER
Division of Biology, California Institute of Technology, Pasadena

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