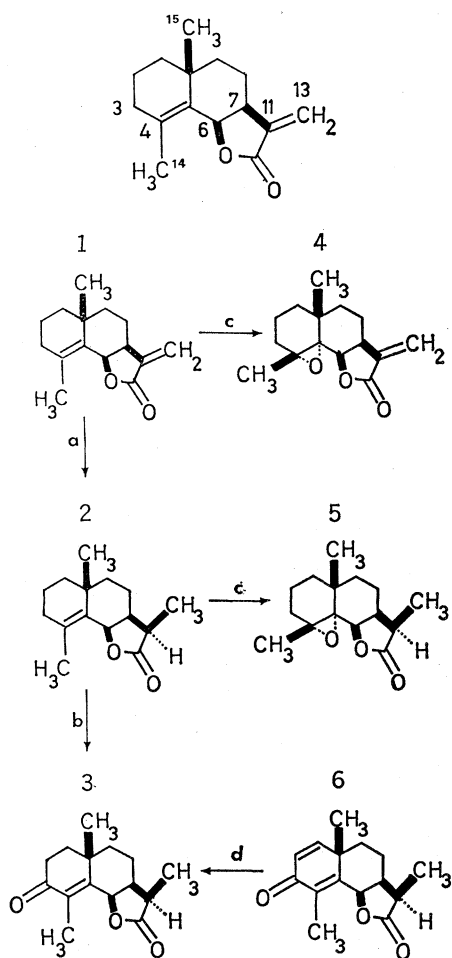




active fraction, which yielded the inactive triterpene ketone friedelin from a benzene solution (about 0.05 percent). The mother liquors were distilled twice at reduced pressure to give, first a more volatile, inactive fraction (probably a sesquiterpene alcohol), and then a less volatile, viscous, active distillate [about 1 percent, boiling point at 0.1 torr at 120° to 130°C (bath temperature)]. This distillate crystallized nearly completely on standing. Recrystallization from hexane gave a highly active substance (about 0.2 percent), melting at 77°C; its specific rotation $[\alpha]_D$ was -113° in CHCl_3 ; its composition was $\text{C}_{15}\text{H}_{20}\text{O}_2$. Its infrared spectrum in KBr showed bands at 1756, 1666, and 1644 cm^{-1} ; the nuclear magnetic resonance spectrum showed signals at chemical shifts (δ , ppm) from tetramethylsilane: 6.18 and 5.60 (H-13); 5.28 (H-6); 2.98 (H-7); 1.76 (H-14), 1.09 (H-15), in CDCl_3 .

Structure 1 was deduced from the spectral properties of the substance itself and of the derivatives prepared by the reactions summarized in Fig. 1. The α,β -unsaturated ketone 3 is identical with 1,2-dihydro-6-epi- β -santonin, prepared from 6-epi- β -santonin 6 (3).

Fig. 1. The reagents used in the transformations shown were as follows. (a) Sodium borohydride and ethyl acetate; (b) sodium chromate and sodium acetate in acetic acid and acetic anhydride; (c) *p*-nitroperbenzoic acid and ethyl acetate; (d) hydrogen, tris-triphenylphosphinorhodium chloride, benzene, and ethanol. All reactions occurred at room temperature. Substances obtained were characterized as follows. Structure 2, m.p. 69°C; infrared $1758, 1645\text{ cm}^{-1}$. Structure 3, m.p. 150°C; $[\alpha]_D -150^\circ$; maximum absorption (ultraviolet) at 245 nm (in ethanol) (molar extinction, 15,100). Structure 4, m.p. 143°C, $[\alpha]_D -69^\circ$. Structure 5, m.p. 183°C. Structure 6, m.p. 193°C. All the substances mentioned gave analytical values within, at most, 0.4 percent of the theoretical values, and other spectral data are in agreement with the structures proposed.

A mixed sample of *Frullania* spp., containing mostly *Fr. tamarisci* (L.) Dum. and *Fr. dilatata* (L.) Dum., collected from rocks in the Dordogne region (August 1968) gave, by the same procedure, a less levorotatory solid distillate (about 1 percent), which on repeated crystallization from hexane gave a pure substance, melting at 95°C, which was optically inactive. This is the racemic mixture ($\pm$) of structure 1 as shown by the identity of nuclear magnetic resonance (NMR) spectra and of infrared spectra in solution and by the slight differences of the infrared spectra in the solid state. The product of epoxidation was similarly proved to be the optically inactive ($\pm$) structure 4.

The isolation from liverworts of eudesmanolides extends the range of distribution of this class of substances, widespread in some tribes of Compositae (4). Our patient gave positive allergic reactions to several sesquiterpene lactones from Compositae (damasin, parthenin), but not to others (α -

santonin, desacetylconfertifolin, psilostachyin B); his sensitivity does not appear to be directly linked with any of the individual functional groups present in structure 1.

It is quite exceptional to find sesquiterpenes belonging to enantiomeric series (5). It may therefore be of more than passing interest to note that another liverwort contains (–)-longifolene, the enantiomer of the common sesquiterpene, (+)-longifolene (6).

Note added in proof: Extraction of homogeneous *Fr. dilatata* (L.) Dum., collected in the Dordogne area on saplings of chestnuts (April 1969) led (with J.-C. Muller) to the isolation of the pure enantiomer of lactone 1, m.p. 76°C, $[\alpha]_D +114^\circ$; this is also allergenically active on our patient.

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 2. We thank Prof. A. Gagnieux, Mrs. N. Ourisson, and Miss A.-M. Lambert for help in locating the plants, in collecting them, and in identifying them.
 3. A sample of 6-epi- β -santonin was provided by Prof. W. Cocker, University of Dublin; it was hydrogenated as indicated in Fig. 1. 1,2-Dihydro-6-epi- β -santonin thus obtained was identical with a sample donated by Prof. E. Piers, University of British Columbia, Vancouver.
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- 26 May 1969

Current-Voltage Relations during Illumination: Photoreceptor Membrane of a Barnacle

Abstract. *In voltage clamped photoreceptor cells of the barnacle, light-induced membrane current varied nonlinearly with membrane potential and changed sign at about +27 millivolts (reversal potential) independently of light intensity. Instantaneous current-voltage relations were linear and intersected the voltage axis at the reversal potential. Illumination increased membrane conductance that was dependent on membrane potential, light intensity, and time.*

Upon illumination, changes in membrane potential of photoreceptor cells occur in several marine arthropod preparations such as the *Limulus* ommatidium (1, 2), the *Limulus* olfactory

nerve (3–5), and the lateral ocellus of the barnacle (6). The positive shift of membrane potential or depolarization due to illumination usually consists of an early transient phase followed

by a steady phase of reduced amplitude. The amplitude of both phases of the response is graded with changes of light intensity. Spike potentials of relatively small amplitude are usually found superimposed upon the slow depolarization in limulus ommatidial cells, whereas spike trains have not been observed to occur in photoreceptors of limulus olfactory nerve or the barnacle ocellus.

Two hypotheses have been proposed in order to interpret the membrane mechanism of the slow potential changes. (i) The potential change of the photoreceptor membrane arises because of a conductance change during illumination, and membrane potential approaches a fixed potential that is independent of the intensity of illumination (2, 7). This concept implies that the potential change can be explained in terms of membrane conductance that is dependent upon light intensity and time. (ii) The potential change occurs because of deactivation by light of a voltage-dependent electrogenic sodium pump (4, 5). Using the voltage clamp technique, we have analyzed the mechanism of potential change elicited by illumination. Although this method has been used extensively to study membrane mechanisms of most classes of excitable tissues, it has never been successively applied to the study of receptor cells, mainly because of the small size of most receptors. The relatively large size (about 80 μm) of photoreceptor cells in the lateral ocellus of the barnacle [*Balanus eburneus* (8)] has allowed us to overcome this difficulty.

The lateral ocellus with its connecting ocellar nerve was isolated and mounted in a recording chamber with the corneal side lying adjacent to a small fiber optics bundle that transmitted light from a shuttered 150-watt tungsten-halide light source (unit intensity approximately 1076 millilamberts); intensity was reduced with neutral density filters. The preparation was perfused continuously with artificial barnacle saline (9). A photoreceptor was penetrated under visual control by two glass microelectrodes (tip diameter $< 1 \mu\text{m}$) filled with 3M KCl. Membrane potential was recorded on a cathode-ray oscilloscope (CRO) as the potential difference between one internal electrode and a KCl-filled microelectrode in the saline bath. The second internal electrode was used to apply a constant current pulse (current clamp method) or to control the membrane potential by a feedback system between the po-

tential and current-passing electrodes (voltage clamp method). The inherent advantage of the latter method is that membrane properties during illumination can be evaluated independently of any secondary changes that may occur as a result of potential changes caused by light. The time required to clamp membrane potential to a final value with the system used was about 2.0 msec when a rectangular commanding voltage pulse was applied to the feedback amplifier. Membrane current was displayed on the CRO as the voltage drop across the feedback resistor of an operational amplifier used to hold the bath at ground potential.

The resting potential of a dark-adapted cell was usually about -40 mv , as measured with respect to the external solution. When light of low intensity was applied (Fig. 1A), the potential change had a more or less rectangular time course; that is, the early transient phase was not clear. As the intensity was increased, the transient phase became more marked, and at unit intensity the membrane potential at the peak of the transient phase exceeded zero potential; membrane potential then reversed its sign. When the membrane potential was "clamped"

near the resting potential (-37 mv), light resulted in an inward current through the membrane (Fig. 1A); the time course of membrane current was qualitatively similar to that of membrane potential because both were approximately rectangular at low light intensity and an early transient phase emerged at higher intensity.

Membrane current initiated by illumination (current with illumination minus that without illumination) was inward at the resting potential, and the amplitude of this current decreased as membrane potential was clamped to more positive levels (third row). Current changed its sign from inward to outward at about $+27 \text{ mv}$; at this membrane potential the light-initiated current was practically zero. This is shown graphically in Fig. 1B, where light-initiated membrane current (ordinate) is plotted as a function of the extrinsically controlled membrane potential (abscissa) for peak and steady membrane currents; inward current and inside negative membrane potential are shown on the negative axes. The current-voltage ($I-V$) relations are non-linear; that is, membrane current, for a unit increment of potential, increased as membrane potential became more

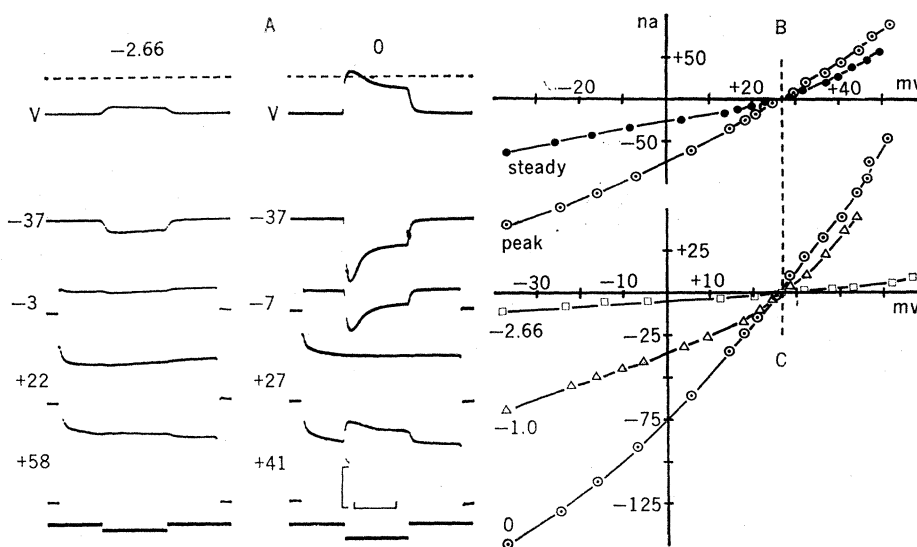


Fig. 1. (A) Records in the top row show the membrane potential change (V) associated with two different intensities of illumination; logarithm of intensity is indicated above each record, and duration of illumination is shown at the bottom of each column of records. Zero membrane potential is indicated by the dashed horizontal lines; negative membrane potential is downward from this line. The remaining records in each column are voltage clamp records showing membrane current associated with the same intensities of illumination. The membrane potential at which each record was obtained is indicated adjacent to each record; inward membrane current is displayed downward. Vertical and horizontal calibration marks: 40 mv, 200 na, and 400 msec. (B) Current-voltage ($I-V$) relations obtained from voltage clamp records at the peak of the transient (double circles) and steady (solid circles) phases of membrane current elicited by light of unit intensity. (C) The $I-V$ relations of the peak light-initiated current for three different intensities of illumination. Intensity expressed as the logarithm of unit intensity adjacent to the appropriate curve.

positive. Although the slope of the relation at any given membrane potential is greater for the peak than it is for the steady phase, the two curves intersect the voltage axis approximately at the same point (dotted line). The membrane potential at this point (reversal potential) was about +27 mv in this cell. The mean reversal potential of 33 cells in normal saline was 26.9 ± 5 mv (S.D.). The slope of an $I-V$ relation at a given membrane potential increased as light intensity increased, but the reversal potential was constant

and therefore independent of light intensity (Fig. 1C). The reversal potential of the steady phase was also independent of light intensity and was the same as the peak phase for all light intensities.

Figure 2 (a different cell) shows an instantaneous $I-V$ relation of the membrane during illumination. The dashed line is the $I-V$ curve for the peak current obtained by the method already described. After this series of measurements, membrane potential was clamped near the resting potential (peak cur-

rent at this level is indicated by the double circle), and relatively brief voltage steps were added to the membrane potential at the time of peak current. Membrane current with (I_2) and without (I_1) illumination was obtained with this procedure. The early time course of light-initiated membrane current ($I_2 - I_1$) during the voltage pulse was calculated as shown in the lower inset. Light-initiated current during the pulse should contain little capacitative current because the capacitative surge was similar with and without illumination and was canceled out by the method used to determine $I_2 - I_1$. Values of $I_2 - I_1$, 2.0 msec after the onset of the voltage pulse, were obtained at different V 's and are plotted in the main graph. The resulting instantaneous $I-V$ relation is almost linear and crosses the potential axis near the reversal potential. Similar results were obtained at several different holding potentials. If at a slightly later time $I_2 - I_1$ is plotted against V , the relation deviates from a straight line and tends to approach the relation represented by the dashed line. At 30 msec after the onset of the voltage pulse, the former almost coincides with the latter. This suggests that the light-sensitive membrane conductance is dependent on membrane potential and has a relaxation time of 30 msec.

To obtain an estimate of the conductance increase associated with light intensity, chord conductances at the resting potential (-38 mv) were calculated from the $I-V$ relations in Fig. 1C. They were, from low to high intensity, 1.6, 9.6, and $20.0 (\times 10^{-7})$ reciprocal ohm for the peak and 1.4, 5.1, and $8.0 (\times 10^{-7})$ reciprocal ohm for the steady phase (some of the steady phase $I-V$ relations are not shown in Fig. 1). The conductance of the cell at the same potential without illumination was 8.7×10^{-7} reciprocal ohm. Thus membrane conductance for the three light intensities increased by factors of 1.2, 2.1, and 3.3 at the peak and 1.2, 1.6, and 1.9 during the steady phase.

These results do not seem to support the hypothesis that depolarization during illumination is caused by alteration of metabolic current, as derived from experiments conducted on *Limulus* photoreceptors where a conductance increase was not detected during light (5). With the voltage clamp technique we have demonstrated increased membrane conductance during illumination

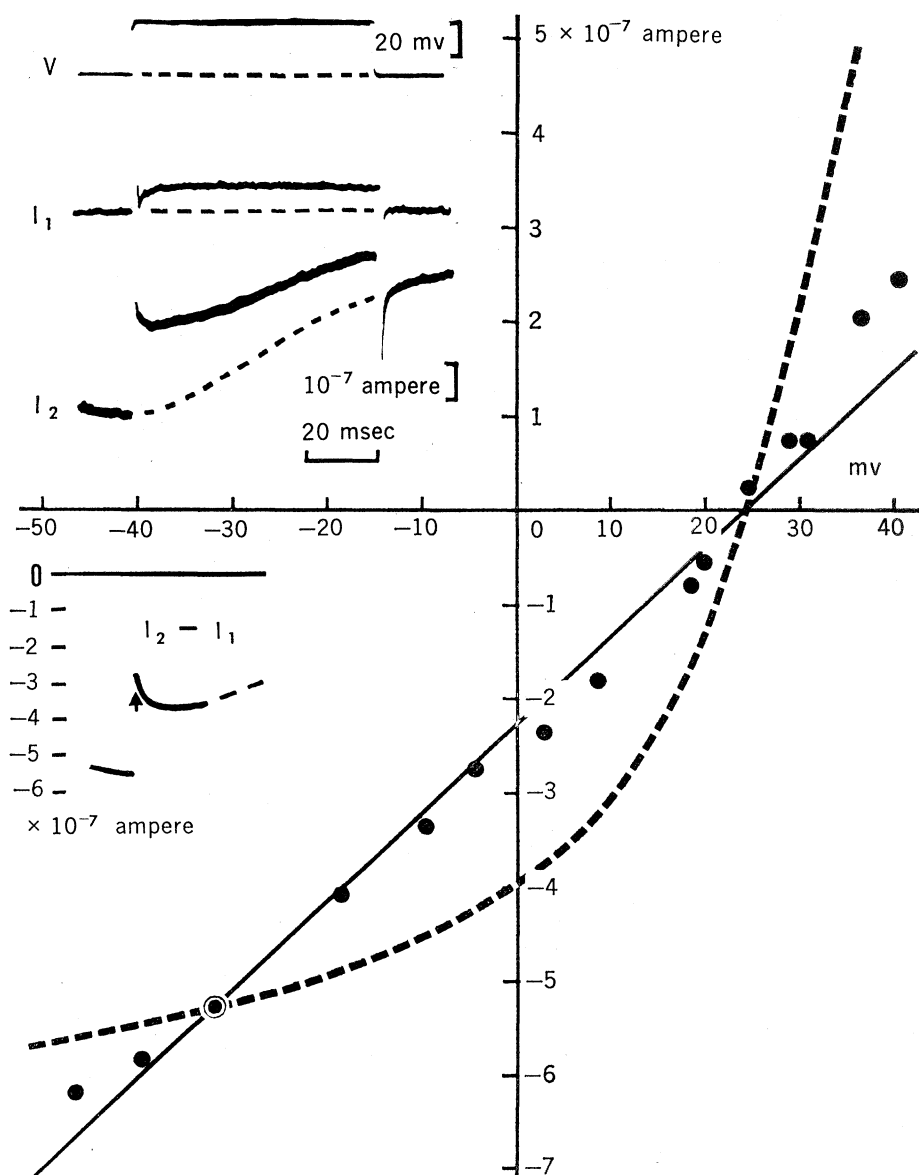


Fig. 2. Instantaneous $I-V$ relation during illumination. (Top inset) Membrane current obtained without illumination (I_1) and with illumination (I_2) during the application of a potential step (V) coinciding in time with the peak of the transient phase. Dashed lines represent records obtained without the added step pulse. (Bottom inset) Early time course of light-initiated membrane current ($I_2 - I_1$) during the step pulse. Values of $I_2 - I_1$ were measured 2.0 msec after the pulse (arrow) and are shown as a function of membrane potential (solid circles) in the main graph; the straight line through membrane current at the holding potential and the reversal potential represents a linear instantaneous $I-V$ relation. The dashed curve represents the $I-V$ relation obtained by the same method depicted in Fig. 1.

and the existence of a reversal potential that is invariant with light intensity, membrane potential, and time. It has been shown that reducing sodium concentration in the bathing solution is associated with a less positive reversal potential in this preparation (10). Therefore, the depolarizing receptor potential seems to be the result of a permeability increase to sodium ions.

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Short Fragments from Both Complementary Strands in the Newly Replicated DNA of Bacteriophage SPP-1

Abstract. *Bacillus subtilis* bacteriophage SPP-1 has separable complementary DNA strands. Fragments of nascent DNA isolated a very short time after phage infection show that these short chains are complementary to both phage DNA strands, as observed by hybridization techniques.

Okazaki *et al.* (1) have isolated newly synthesized DNA as short segments from both *Escherichia coli* and those infected with bacteriophages. These segments had a molecular weight of 1 to 2×10^6 daltons, as judged by sedimentation behavior in alkaline sucrose gradients. The nascent DNA was labeled by an incorporated radioactive tracer to which the cells were exposed for short intervals. As the duration of the labeling was increased, more of the labeled DNA was found in larger molecules. These results, together with the observations on the action of ligases (2), have prompted reexamination of discontinuous models of DNA replication (3).

In these experiments it is difficult to rule out the possibility that the fragments might have been the result of nuclease or shear action on the sensitive nascent DNA (4). Regardless of whether the fragments of nascent DNA are true intermediates in DNA biosynthesis or products of degradation, one can ask if they are complementary to one or both strands of the DNA template. The results of the following hybridization experiments with *Bacillus subtilis* bacteriophage SPP-1, whose complementary strands are readily separable, suggest that the fragments of nascent DNA are complementary to both strands of the phage DNA.

Bacteriophage SPP-1 was isolated by Riva *et al.* (5). Infection, growth, purification of phage DNA, and separation of the DNA strands were carried out

as described (5). The phage has a double-stranded DNA molecule of 2.5×10^7 daltons, with a density of 1.703 g/cm^3 (in CsCl). The profile of separated strands in CsCl is shown in Fig. 1. Phage infection was carried out with an arginine-requiring *B. subtilis* (SB 1051) as host. Stationary phase cells were infected at 37°C and transferred to 30°C after 40 minutes. At specified intervals of phage growth, portions were taken and added to tubes containing H^3 -thymidine. After exposure to tracer for the desired time (usually a few seconds), growth was stopped by the addition of ice-cold sodium azide until the final concentration was 0.01 mole/liter . The cells were washed after 10 minutes with $0.01M$ tris-HCl [tris-(hydroxymethyl)aminomethane] buffer, $\text{pH } 7.5$, containing $0.01M$ sodium azide; and they were then frozen. The killed frozen cells were thawed, and lysozyme was added to a final concentration of $100 \text{ } \mu\text{g/ml}$. After 2 minutes at 37°C , NaOH was added to a final concentration of $0.01M$. The cleared lysate was subjected to zone centrifugation in an alkaline sucrose gradient (5 to 20 percent). Fractions were collected, and samples were neutralized and analyzed for radioactivity in the acid-precipitable portions.

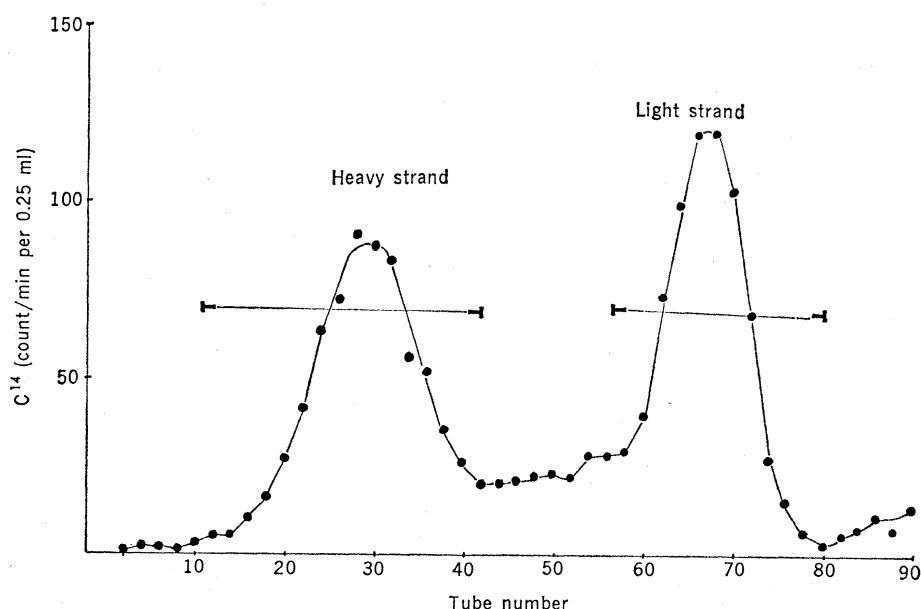


Fig. 1. Separation of complementary strands. Purified phage DNA labeled with C^{14} -thymidine ($226 \text{ } \mu\text{g}$; $120 \text{ count min}^{-1} \mu\text{g}^{-1}$) in 0.8 ml of $0.2 \times \text{SSC}$ and $50 \text{ } \mu\text{l}$ of a solution containing $445 \text{ } \mu\text{g}$ of *B. subtilis* ribosomal RNA were mixed with 12 ml of $0.2 \times \text{SSC}$ in a 50-ml flask, heated for 10 minutes at 100°C , and chilled in ice. Solid CsCl was added to give a refractive index of 1.4030 . Centrifugation was performed in a Spinco model-L centrifuge (rotor 50) at $32,000 \text{ rev/min}$ for 64 hours at 20°C . From every other fraction of the first three gradients, $25 \text{ } \mu\text{l}$ were taken and used for counting the radioactivity. The regions indicated on top of the heavy and light strand strata represent the pooled fractions from three gradients. SSC, standard saline-citrate solution ($0.15M \text{ NaCl} + 0.015M \text{ sodium citrate}$).