[4] Rabinovitch, M., L. C. U. Jungqueira, and H. A. Rothschild, *Science*, **114**, 551 (1951).
[5] Kassenaar, A., A. Kouwenhoven, and A. Querido, *Acta Endocrinol.*, **39**, 223 (1962).
[6] Kochakian, C. D., and D. G. Harrison, *Endocrinology*, **70**, 99 (1962).
[7] Mueller, G. C., A. M. Herranen, and K. F. Jervell, *Recent Prog. Hormone Research*, **14**, 95 (1958).
[8] Mueller, G. C., J. Gorski, and Y. Aizawa, these PROCEEDINGS, **47**, 164 (1961).
[9] Wilson, J. D., *J. Clin. Invest.*, **41**, 153 (1962).
[10] Kalman, S. M., and P. M. Opsahl, *Biochim. Biophys. Acta*, **49**, 614 (1961).
[11] Wilson, J. D., *Biochem. Biophys. Res. Comm.*, **8**, 175 (1962).
[12] Hamilton, T. H., these PROCEEDINGS, **49**, 373 (1963).
[13] Liao, S., and H. G. Williams-Ashman, these PROCEEDINGS, **48**, 1956 (1962).
[14] Perry, R. P., these PROCEEDINGS, **48**, 2179 (1962).
[15] Kirby, K. S., *Biochem. J.*, **64**, 405 (1956).
[16] Hoagland, M. B., M. L. Stephenson, J. F. Scott, L. I. Hecht, and P. C. Zamecnik, *J. Biol. Chem.*, **231**, 241 (1958).
[17] Britten, R. J., and R. B. Roberts, *Science*, **131**, 32 (1960).
[18] Szybalski, W., *Experientia*, **16**, 164 (1960).
[19] Bray, G. A., *Analytical Biochem.*, **1**, 279 (1960).
[20] Keller, E. B., and P. C. Zamecnik, *J. Biol. Chem.*, **221**, 45 (1956).
[21] Maxwell, E. S., these PROCEEDINGS, **48**, 1639 (1962).
[22] Tepperman, J., *Metabolic and Endocrine Physiology* (Chicago: Year Book Medical Publishers, 1962), p. 57.
[23] Nirenberg, M. W., and J. H. Matthaei, these PROCEEDINGS, **47**, 1588 (1961).
[24] Barondes, S. H., C. W. Dingman, and M. B. Sporn, *Nature*, **196**, 145 (1962).
[25] Singer, M. F., O. W. Jones, and M. W. Nirenberg, these PROCEEDINGS, **49**, 392 (1963).

---

*COUPLED PHOTOREDUCTION OF UBIQUINONE AND PHOTOOXIDATION OF FERROCYTOCHROME C CATALYZED BY CHROMATOPHORES OF RHODOSPIRILLUM RUBRUM**, †

BY W. S. ZAUGG

CHARLES F. KETTERING RESEARCH LABORATORY, YELLOW SPRINGS, OHIO

Communicated by Martin D. Kamen, May 27, 1963

A light-induced reaction between bacteriochlorophyll and a cytochrome of type *c* in *Chromatium* cells has been reported by Chance and Nishimura,[1] who showed a temperature-independent oxidation (down to 80°K) of the cytochrome under anaerobic conditions at a rate reported to be 0.39 μM Fe^{++}/sec. Duysens[2] had earlier described a light-induced oxidation of cytochrome in *Rhodospirillum rubrum* under anaerobic conditions. In the experiments of Duysens, as well as in those of Chance and Nishimura, cytochrome was again reduced in the dark. Clayton[3] showed that the light-induced oxidation of cytochrome in *Chromatium* chromatophores is closely connected to the absorption changes of a special component of bacteriochlorophyll. Evidence to date therefore favors a cytochrome as the electron donor reacting directly with bacteriochlorophyll upon illumination.

Ubiquinone (UQ)† has been implicated as a primary electron acceptor in bacterial photosynthesis by Clayton,[4] who related the light-induced absorption changes in the 240–350 mμ range to the reduction of UQ in chromatophores from *Chromatium*, *R. rubrum*, and *Rps. spheroides*. He also showed there was almost a 1:1 relation-

ship between the cytochrome oxidized and the UQ reduced in the light reaction, if the absorbancy change at 280 mμ could be attributed to the quinone. Bales and Vernon[5] have correlated the rapid photooxidation of reduced 2,6-dichlorophenolindophenol by chromatophores of *R. rubrum* with absorption changes at 280 mμ, implicating UQ as the electron acceptor in this reaction.

Vernon and Kamen[6] reported a photooxidation of added ferrocytochrome *c* by *R. rubrum* chromatophores in the presence of oxygen. Vernon[7] later demonstrated that this reaction could take place under strict anaerobicity upon the addition of fumarate or NAD. Smith[8] concluded that the photooxidation of added cytochrome *c* was not enzymic since chromatophores which had been heated at 70° for 30 min still exhibited the reaction, even though proteins of the extract were precipitated.

Lindstrom[9] studied the photooxidase activity of chromatophores using reduced 2,6-dichlorophenolindophenol as the electron donor, and concluded that such photooxidations are valid measurements for the oxidant in bacterial photosynthesis.

The experiments reported here show a very rapid photooxidation of added mammalian cytochrome *c* by chromatophores of *R. rubrum* with added UQ as the electron acceptor. Reduced UQ reduces the oxidized cytochrome *c* both chemically and enzymically in the dark, making the system reversible.

Methods and Materials.—*R. rubrum* cells were grown in a medium containing malate, glutamate, acetate, and ammonium chloride, as described previously.[10] Chromatophores were prepared by 2 min sonic oscillation (Raytheon, 10 kc) of the whole cells, followed with two washings by centrifugation of the particles sedimenting from 20,000–100,000 × *g*.[10]

Reactions were followed in a modified Beckman DB spectrophotometer. A cover for the cell compartment was constructed having a square opening, 14 mm on a side, directly above the sample cell. A square tube attached to the under side of the cover funneled the light directly to the sample, thereby eliminating stray light from reaching the phototube. In addition, cuvettes containing 1 F $CuSO_4$ were placed between the sample (and reference) and the phototube to minimize any effect of scattered light. The light source was an American Optical Universal microscope lamp placed 12 cm above and focused on the 1 ml sample. The lamp used a 6.5 v, 2.75 amp clear glass bulb and the variable voltage selector was set at 7.5 v, except where otherwise stated. The light beam passed through 2 cm of water and a Corning glass filter No. 2403 before passing through the opening in the cover of the cell compartment. Chlorophyll was determined by the method of van Niel and Arnold.[11] Reduced quinone was prepared according to the procedure of Green and Burkhard.[12] Horse heart cytochrome *c* obtained from Sigma Chemical Co. was used in these experiments. The experiments reported here were carried out aerobically, since the degree of anaerobicity had no effect on the rate of the reaction. The rate of oxygen-dependent cytochrome *c* photooxidation by *R. rubrum* chromatophores, which was reported by Vernon and Kamen,[6] is much slower than the quinone coupled photooxidation.

Results.—Figure 1 shows the reduction of cytochrome *c* which occurred when reduced UQ_2 was mixed with cytochrome *c* and *R. rubrum* chromatophores. Subsequent illumination produced an immediate oxidation of the cytochrome. The sequence could then be repeated by turning off the light, waiting until the cytochrome was completely reduced, and again illuminating. After the reaction had

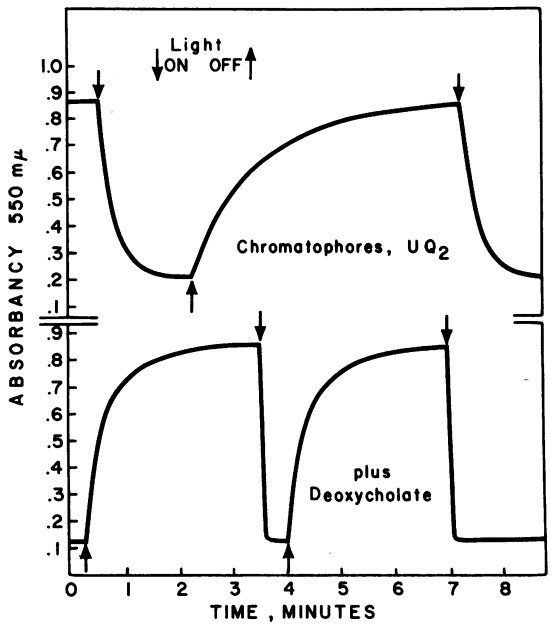

FIG. 1.—Photooxidation of cytochrome *c* by chromatophores of *R. rubrum* in the presence of ubiquinone. The reaction mixture consisted of 0.47 mmoles sucrose, 0.03 mmoles phosphate buffer (final pH 7.0), 9.0 μmoles KCl, 36 mμmoles cytochrome *c*, 110 mμmoles reduced UQ_2 added in 0.01 ml ethanol,and 0.01 ml *R. rubrum* chromatophore preparation containing 0.011 mg bacteriochlorophyll in a final volume of 1.0 ml. The reaction was followed at 550 mμ in a modified Beckman DB spectrophotometer with external recorder. The cell compartment was maintained at 25°C with circulating water. At the points indicated in the upper curve, the light was turned on, resulting in an oxidation of the cytochrome *c* which had previously been reduced by the reduced UQ_2. The lower curve shows the effect of adding 0.02 ml of a 10% solution of sodium deoxycholate to the above reaction mixture. The initial portion of this curve shows the reduction of cytochrome *c* by reduced UQ_2 in the dark. These experiments were performed under aerobic conditions. However, as discussed in the *Methods* section, the degree of anaerobicity did not affect the rate of cytochrome photooxidation.

been allowed to complete two cycles, the reaction mixture (1 ml) was made 0.2 per cent in deoxycholate by addition of 0.002 ml of a 10 per cent solution. Presence of the bile salt increased the rates of both the dark and light reactions. From other experiments it appeared that the detergent acted not only by increasing the solubility of UQ, but also by making the reaction site on the chromatophore more available to the reactants. As expected, the effect of deoxycholate was more pronounced when UQ_6 and UQ_{10} were used, since in the absence of the bile salt both the dark and light reactions were comparatively slow.

The rates of the photoreactions in deoxycholate-treated chromatophores, using UQ_6 or UQ_2, generally ranged from 6–8 mmoles cytochrome *c* oxidized/hr/mg chlorophyll, with one series of reactions averaging 9 mmoles/hr/mg chlorophyll. This rate exceeds by more than fivefold, on an equivalent electron basis, the value obtained by Vernon[13] for the photooxidation of reduced 2, 6-dichlorophenolindophenol in the presence of NAD (0.553 mmole/hr/mg chlorophyll).

Figure 2 shows the dependence of the photooxidation of reduced cytochrome *c* upon the presence of UQ_6. The first curve in this figure shows that chromatophores, in the presence of cytochrome *c* (a small amount of which is already in the reduced form) and 0.2 per cent deoxycholate, are capable of rapidly photooxidizing a small amount of cytochrome in the absence of added UQ. The amount of cytochrome oxidized is about 3.8 mμmoles/ml. If this corresponds to endogenous quinone reduced, the photoreaction results in the reduction of 1.9 mμmoles/ml of UQ_6. Since there were 11.4 mμmoles/ml of bacteriochlorophyll in the reaction mixture, the ratio of chlorophyll to quinone would be about 6 to 1 in this preparation of *R. rubrum* chromatophores. There is at present no report in the literature which compares the amount of UQ in *R. rubrum* chromatophores directly with chlorophyll

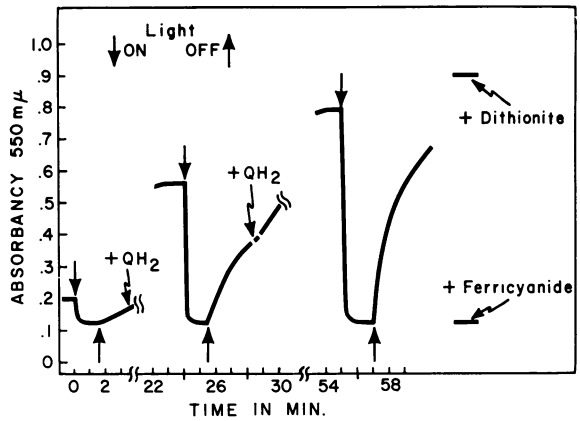

FIG. 2.—Dependence of cytochrome *c* photooxidation on added UQ. The reaction mixture contained 0.47 mmoles sucrose, 9.0 μmoles KCl, 0.03 mmoles phosphate buffer (final pH 7.0), 41 mμmoles cytochrome *c*, 0.02 ml of 10% sodium deoxycholate solution, and 0.01 ml *R. rubrum* chromatophores containing 0.011 mg bacteriochlorophyll in a volume of 1.0 ml. At places indicated, 11 mμmoles of reduced UQ_6 were added in 0.001 ml ethanol. The reaction was followed at 550 mμ as indicated in Fig. 1. See text for explanations.

content. Vernon[13] used the results of three separate investigations on content of UQ,[14, 15] cytochrome, and chlorophyll[16] to arrive at an approximate value of 3 chlorophylls to 1 UQ for *R. rubrum*. The first addition of 11 mμmoles of reduced UQ resulted in a rather slow reduction of cytochrome *c* which required about 18 min to reach completion. The sample was then illuminated (center curve of Fig. 2), resulting in the oxidation of 20 mμmoles of cytochrome *c* as determined by the change in absorbancy at 550 mμ, using a millimolar absorbancy index of 21.0.[17] After turning off the light and allowing the dark reduction of cytochrome *c* to proceed for a few minutes, a second aliquot of reduced UQ_6 was added (11 mμmoles). A greater quantity of cytochrome then became reduced, which was completely oxidized upon illumination (third curve).

The apparent discrepancy in the ratio of cytochrome photooxidized to quinone added, shown in the third curve of Figure 2 (31:22), compared to that shown by the center curve (20:11), is most likely due to oxidation of the reduced quinone by oxygen in the air. This was indicated by other experiments in which limiting amounts of UQ were added. The amount of cytochrome *c* photooxidized gradually decreased through several cycles. With an excess of reduced quinone, however, the light-dark cycle could be repeated many times during a 2–3 hr period without appreciable change in the light-dependent reaction. The dark reaction tended to become slower, however, because of the decrease in reduced UQ-cytochrome *c* reductase activity. It can be noted by the absorbancy reading in the presence of ferricyanide (cytochrome *c* completely oxidized) that the light-induced reaction with excess quinone resulted in complete oxidation of the added cytochrome.

Evidence that the added quinone was reduced during the photooxidation of cytochrome was obtained from measurements in the 260–290 mμ region. Figure 3 shows the absorption spectra of oxidized UQ_2 obtained in the dark (curve *A*) and reduced UQ_2 produced in the light (curve *B*). Almost identical curves were obtained upon repeating the light-dark sequence on the same sample.

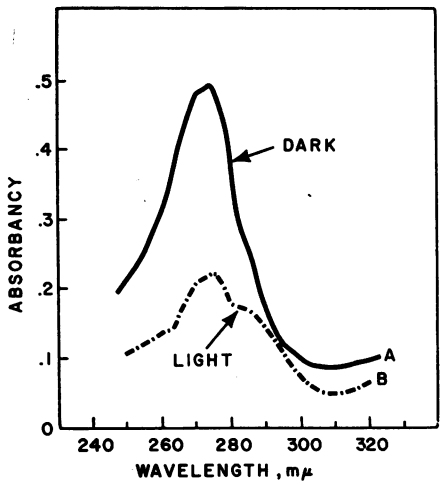

FIG. 3.—Spectral evidence for UQ photoreduction. A 6 ml reaction mixture was made up containing the same final concentrations of sucrose, KCl, phosphate, cytochrome *c*, and deoxycholate as mentioned in Fig. 2. The final concentration of *R. rubrum* chromatophores corresponded to a bacteriochlorophyll concentration of 0.012 mg/ml. A 1 ml sample of this reaction mixture was withdrawn and placed in a cuvette in the sample compartment of the spectrophotometer. The remainder of the mixture was divided equally between two cuvettes to be used as references. One reference cell contained cytochrome *c* in the oxidized form while the cytochrome in the other reference cell was reduced with $NaBH_4$. To the sample cell was added 22 mμmoles of reduced coenzyme Q_2 in 0.002 ml of ethanol. After waiting a few minutes for the cytochrome *c* to become reduced, the light was turned on. With the light on (cytochrome *c* oxidized, quinone reduced) a difference spectrum was taken against the reference containing oxidized cytochrome *c* (curve *B*). A check at 550 mμ showed that the degree of oxidation in the reference cell was essentially the same as in the illuminated sample cell. The light was then turned off and, after reduction of the cytochrome by the reduced quinone, a difference spectrum was taken against the reference cell containing reduced cytochrome *c* (curve *A*). A second illumination (again using the reference cell containing oxidized cytochrome *c*) resulted in a difference spectrum which was the same as curve *B*. For these experiments it was necessary to filter the incident light (variable voltage selector set at 4.0 volts; see *Methods* section) through two Corning glass filters (numbers 2403 and 2550) and 2 cm of water, and also to use 1 cm of water as a barrier filter between the illuminated sample and phototube to minimize any effect of scattered light on the spectra taken in the UV region during illumination.

Curve *B* shows the presence of some oxidized quinone in the light. Although cytochrome and quinone were added in approximately equivalent amounts (2 molecules of cytochrome to 1 molecule of quinone), it is apparent that in this experiment not all the quinone was originally in the reduced form. However, the curves shown were taken within a few minutes of each other, and definitely show the changes in redox state of quinone which accompany cytochrome *c* photooxidation.

In agreement with previous observations on the heat stability of the photooxidation reactions of *R. rubrum* chromatophores [8, 9] it was found that good activity remained in chromatophores which had been incubated at 60° for 3 hr. However, when heated to 90° for 2 min no activity could be observed even after sonication of the heated particles.

Discussion.—The rapid photoreaction reported here, involving the oxidation of added cytochrome *c* and a simultaneous reduction of added UQ by *R. rubrum* chromatophores, is a model system for the primary events currently believed to take place when whole cells are illuminated. Investigators have shown that photooxidation of a *c* type cytochrome occurs very early in the sequence of changes caused by illumination of cells as well as chromatophores of photosynthetic bacteria.[1, 18–22] Quinone has been implicated as an electron acceptor in the photooxidation of reduced 2,6-dichlorophenolindophenol,[5] and Clayton[4] has proposed that UQ acts as a primary electron acceptor in bacterial photosynthesis. The demonstration that chromatophores are capable of the rapid photoreaction described in this paper lends support to the current concept of cytochrome and quinone function in the primary photochemical event.

The ability of chromatophores to carry out the reduction of a quinone by the same chlorophyll system which oxidizes a cytochrome may well represent one of the

fundamental differences between the bacterial system and that of higher plants. The current evidence indicates that quinone in the plant system is reduced by a different chlorophyll system than that which oxidizes the cytochrome.[23] This could account for the observation that *R. rubrum* chromatophores demonstrate a true cyclic photophosphorylation, while chloroplasts do not perform this function at an appreciable rate, unless phenazine methosulfate is added to serve as a cofactor. Attempts thus far to show a rapid photooxidation of cytochrome *c* and a coupled reduction of UQ or plastoquinone in chloroplasts or chloroplast fragments under a variety of conditions have not been successful, further demonstrating a basic difference between the two photosynthetic systems.

The observation of reduced UQ-cytochrome *c* reductase activity in *R. rubrum* chromatophores, which experiments now in progress show is antimycin-sensitive, furnishes evidence of a dark electron transfer system similar to that of mitochondria. If a comparison can be made between the sites of phosphorylation in the two systems, ATP formation would be expected to occur during the transfer of electrons from the reduced quinone to the *c* type cytochrome in bacteria. The photooxidation by *R. rubrum* chromatophores of added cytochrome *c* with the simultaneous photoreduction of added UQ and the subsequent re-reduction of cytochrome *c* in a dark, antimycin-sensitive reaction may very well represent the flow of electrons which results in cyclic photophosphorylation.

The author wishes to thank Dr. L. P. Vernon for his comments and suggestions, and Ann Tirpack for her assistance during the course of the investigation. UQ_2 was a gift of Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey.

* Contribution No. 117 of the Charles F. Kettering Research Laboratory.

† The term "chromatophore" designates the photosynthetically active particle or fragment which is obtained upon rupture of the intact cell by sonic oscillation. The name Ubiquinone (UQ) is used as suggested by the Commission on Enzymes of the International Union of Biochemistry.[24]

[1] Chance, B., and M. Nishimura, these PROCEEDINGS, **46**, 19 (1959).

[2] Duysens, L. N. M., *Nature*, **173**, 692 (1954).

[3] Clayton, R. K., *Photochem. Photobiol.*, **1**, 313 (1962).

[4] Clayton, R. K., *Biochem. Biophys. Res. Comm.*, **9**, 49 (1962).

[5] Bales, H., and L. P. Vernon, in *Bacterial Photosynthesis*, ed. H. Gest, A. San Pietro, and L. Vernon (Yellow Springs, Ohio: Antioch Press, 1963), in press.

[6] Vernon, L. P., and M. D. Kamen, *Arch. Biochem. Biophys.*, **44**, 298 (1953).

[7] Vernon, L. P., *J. Biol. Chem.*, **234**, 1883 (1959).

[8] Smith, L., *J. Biol. Chem.*, **234**, 1571 (1958).

[9] Lindstrom, E. S., *Plant Physiol.*, **37**, 127 (1962).

[10] Vernon, L. P., *Plant Cell Physiol. (Tokyo)*, in press.

[11] van Niel, C. B., and W. Arnold, *Enzymologia*, **5**, 244 (1938).

[12] Green, D. E., and R. K. Burkhard, *Arch. Biochem. Biophys.*, **92**, 312 (1961).

[13] Vernon, L. P., in *Bacterial Photosynthesis*, ed. H. Gest, A. San Pietro, and L. Vernon (Yellow Springs, Ohio: Antioch Press, 1963), in press.

[14] Lester, R. L., and F. L. Crane, *J. Biol. Chem.*, **234**, 2169 (1959).

[15] Geller, D. M., *J. Biol. Chem.*, **237**, 2947 (1962).

[16] Nishimura, M., *Biochim. Biophys. Acta*, **59**, 183 (1962).

[17] Massey, V., *Biochim. Biophys. Acta*, **34**, 255 (1959).

[18] Chance, B., *Science*, **120**, 767 (1954).

[19] Chance, B., and L. Smith, *Nature*, **175**, 803 (1955).

[20] Olson, J. M., and B. Chance, *Arch. Biochem. Biophys.*, **88**, 26 (1960).

[21] Duysens, L. N. M., *Nature*, **173**, 692 (1954).

[22] Goedheer, J. C., *Biochim. Biophys. Acta*, **38**, 389 (1960).
[23] Witt, H. T., A. Muller, and B. Rumberg, *Nature*, **197**, 987 (1963).
[24] Thompson, R. H. S., *Science*, **137**, 405 (1962).

---

*EVIDENCE FOR MESSAGE READING FROM A UNIQUE STRAND OF PNEUMOCOCCAL DNA**,*†

BY W. R. GUILD AND M. ROBISON

DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER

Communicated by Norman Davidson, May 22, 1963

DNA banded in CsCl gradients at pH 12 has an increased buoyant density due to binding Cs^+ ions to guanine (G) and thymine (T) residues, which ionize when the DNA denatures near pH 11.[1] In the course of other experiments we observed that pneumococcal DNA had an unusually broad and rather flat-topped profile under these conditions, as compared to its denatured band at neutral pH (Fig. 1).

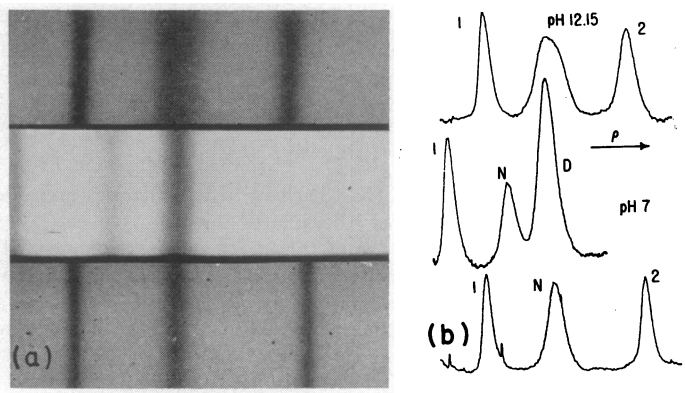

FIG. 1.—Ultraviolet photographs and densitometer tracings of typical pneumococcal DNA bands in CsCl, with reference bands of dAT polymer (1) and *M. lysodeikticus* DNA (2). *Top:* pH 12.15; *middle:* dAT plus native and heat-denatured pneumococcal DNA, pH 7; *bottom:* native, pH 7. Centrifuged 21 hr at 44,770 rpm, 25.0°C. The dAT and *M. lysodeikticus* bands at pH 12 are still rather sharp, whereas the pneumococcal band suggests overlapping peaks. The widths at half maximum are in the ratio 1:1.2:1.9 for native, denatured at pH 7, and pH 12, respectively. Tracings were drawn directly on the figure by a Joyce-Loebl microdensitometer.

Although the mole fraction of G + T equals 0.50 in double-stranded DNA, they need not be equally distributed between the complements. Vinograd *et al.*[1] looked for evidence of strand fractionation in *E. coli*, *M. lysodeikticus*, and T-4 phage DNAs, but found none. Our results generally agree with theirs, in that the effect is certainly small in most species. We have, however, studied the pneumococcal case further, and the results appear to be of significance regarding strand separation,[2] transformation by denatured DNA,[3, 4] and message transcription.

Pneumococcal-transforming DNA carrying four drug resistance markers (streptomycin—S; erythromycin—E; bryamycin—B; and novobiocin—N) was banded in CsCl (A. D. McKay, Inc.) in the SW-39 rotor of the preparative ultracentrifuge at 35,000 rpm for 4 days, as described previously.[3, 5] The pH was adjusted by addition of NH_4OH and NaOH to a value of pH 12.5 ± 0.3, as read on a radiometer TTT-1 pH meter. Bands were fractionated by puncturing the tube and collecting 0.008 ml drops, from one to three per fraction in various experiments.