A COMPARISON OF THE NUMBER OF NUCLEOTIDES PER UNIT LENGTH IN *ESCHERICHIA COLI* AND PHAGE T_4 CHROMOSOMES

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ABSTRACT The grain density produced in radioautographs by fully labeled bacteriophage T_4 chromosomes was compared with the grain densities produced by fully labeled and half-labeled exponential phase *Escherichia coli* chromosomes. Taking into account the differing molar proportions of thymidine in *E. coli* and T_4 chromosomes, it was seen that the two types of chromosomes have approximately equal numbers of nucleotides per unit length, indicating that their structures are the same. Using molecular weight estimates in the literature for the T_4 and related T_2 chromosomes, and the lengths obtained in the radioautographs, the T_4 chromosome was estimated to have between 1.9 and 3.6 nucleotides/3.4 A. On the basis of these values alone, the *E. coli* and T_4 chromosomes could be either one Watson-Crick helix in a form about equal to or more condensed than the B form, or two helices more stretched out than the B form. The length of the T_4 chromosome was 48.7 $\pm$ 4.1 μ when dried on the dull side of Millipore membranes and 42.3 $\pm$ 4.8 μ on the shiny side, under the conditions used. Thus, the supporting surface apparently affects the configuration of a chromosome. Further evidence is also presented in support of the conclusion that the *E. coli* chromosome undergoes semiconservative replication.

INTRODUCTION

Although it has been widely assumed that the *E. coli* and phage T_4 chromosomes consist of two-stranded Watson-Crick helices, the evidence in support of this assumption is somewhat weak. The main work on the *E. coli* chromosome has been that of Cairns (1963 *a*, *b*), of Baldwin and Shooter (1963), and of Cavalieri and coworkers (Cavalieri and Rosenberg, 1961 *a*, *b*, *c*; Cavalieri, Finston, and Rosenberg, 1961; Hall and Cavalieri, 1961).

Cairns (1963 *a*) attempted to show that the *E. coli* chromosome was only one helix by comparing radioautographs of half-labeled *E. coli* chromosomes, having

approximately 1 grain/μ, with radioautographs of fully labeled phage T_2 and phage λ chromosomes. From data presented in a figure (Cairns, 1962), one can calculate that the T_2 and λ chromosomes had 2.4 and 2.8 grains/μ, respectively. Assuming that the T_2 and λ chromosomes were two-stranded, he concluded from this comparison that the *E. coli* chromosome was also two-stranded. As discussed in the present work, however, grain counts of over 1 grain/μ cannot be made accurately, due to coincidence of grains, and grain counts of 2.4 and 2.8 most probably represent actual values of over 4 grains/μ. Thus his grain counts in the *E. coli* and the phage chromosome radioautographs are quite different from what might be expected if all sets of chromosomes were two-stranded. These large differences may be due to variability in the sensitivity of the AR-10 radioautographic film, as was encountered in the present work, or to one or more of the physical conditions which were not kept constant in the separate *E. coli* and phage experiments, including differences in specific activity of the tritiated thymidine, salt concentrations of the suspending media, methods of extraction, and surfaces on which chromosomes were deposited. Therefore, one cannot make quantitative comparisons of the "strandedness" of *E. coli* and phage chromosomes from Cairns' work.

The experiments of Baldwin and Shooter (1963), based on the melting characteristics of 5-bromouracil–labeled hybrid *E. coli* DNA imply that the conserved subunit in DNA replication is the single strand. When taken together with the work of Meselson and Stahl (1958), which showed that only two conserved subunits are present in *E. coli* DNA, their work implies that *E. coli* DNA contains only two strands.

On the other hand, the work of Cavalieri and coworkers (Cavalieri and Rosenberg, 1961 *a*, *b*, *c*; Cavalieri, Finston, and Rosenberg, 1961; Hall and Cavalieri, 1961) using DNase scission, X-ray scission, and electron microscopic observation, indicates that the chromosomes of exponentially growing bacterial cells, including those of *E. coli*, are a pair of double helices. If this work is taken together with that of Meselson and Stahl it would imply that the conserved unit in DNA replication is an intact double helix.

An attempt was made by Josse and Eigner (1966) to resolve the conflicting evidence of Baldwin and Shooter and of Cavalieri and coworkers on the nature of the *E. coli* chromosome. Josse and Eigner presented an extensive two-page set of criticisms of the work of Cavalieri and coworkers. Unfortunately, their critical arguments, except for those based on the Baldwin and Shooter experiments, seem very insubstantial when closely examined. Thus, for example, they state on p. 824, ". . . small-angle x-ray scattering studies have ruled out the possibility of a four-stranded model for isolated bacterial DNA (Luzzati, Luzzati, and Masson, 1962)." However, as Luzzati, Luzzati, and Masson say ". . . it is shown that the x-ray observations could in fact be reconciled with Cavalieri's experiments if it were assumed that the two 'unitary' molecules are loosely associated in the 'biunial' molecule . . ." As another example, Josse and Eigner state: "Autoradiographic analyses of chro-

mosome exchanges in higher plants indicate, however, that the conserved subunit is a single polynucleotide chain (Taylor, 1963). . . ." However they fail to mention that in other radioautographic analyses of chromosome exchanges other patterns of label distribution, called "iso-labeling" are observed (Peacock, 1963; Wahlen, 1965). As pointed out by Wahlen, interpreting this data ". . . does not allow for discrimination between single-helix (unineme) semi-conservative and multihelix (polyneme) conservative replication."

Since the strandedness of the *E. coli* chromosome had not been resolved on the basis of currently available data, further work was undertaken on *E. coli* DNA structure. The experiment used was a comparison of the *E. coli* chromosome to the phage T_4 chromosome by the radioautographic technique of Cairns, using well-controlled conditions to obtain a quantitative relationship of the relative numbers of nucleotides per unit length of the two kinds of chromosomes. The phage T_4 chromosome was chosen as the standard of comparison because considerable work had been performed on it to establish its molecular weight by many independent laboratories using different techniques (see Table II). The experiments bearing on the strandedness of the phage T_4 chromosome have been indirect but the results are consistent with it being a single Watson-Crick helix. Thus, Beer and Zobel (1961) found that the related T_2 chromosome was symmetrical and about 20–25 A in diameter and Cavalieri and Rosenberg (1961 *a*) found that the T_4 chromosomes had melting characteristics like those found for other chromosomes determined to be two-stranded by scission kinetics.

It was found in the present work that the *E. coli* and T_4 chromosomes have nearly equal numbers of nucleotides per unit length, indicating that their structures are the same. If it can be accepted that the T_4 chromosome is two-stranded, then these results imply that the *E. coli* chromosome is also two-stranded.

MATERIALS AND METHODS

Strains and Cultural Media

E. coli B3 and bacteriophage T_4Boltd8, thymine-requiring strains (Simon and Tessman, 1963), were kindly supplied by E. Simon. *E. coli* S/6/5, used in plating for phage titers, was obtained from R. S. Edgar. The minimal medium used was F medium. (Adams, 1959). For complex media, Hershey broth, EHA-bottom agar, and EHA-top agar were prepared as in Steinberg and Edgar (1962). Two experiments were performed. In experiment 1, thymidine-methyl-^{3}H of specific activity 15.7 (New England Nuclear Corp., Boston, Mass.) was added to F medium at 6 μg/ml. In experiment 2, the specific activity was 12.4 and the concentration was 3.5 μg/ml. Suspending medium for *E. coli* B3 stocks contained 50–100 μg/ml thymidine.

Cell Density

Estimates of cell density in liquid culture were made with a Petroff-Hauser chamber. For cells in chain formation, cell equivalent estimates were probably accurate within a factor of two.

Dialysis Cups

The most suitable dialysis cups were made from hollow polyethylene stoppers with a pipetting hole cut in the side and to which 50-mμ pore size Millipore filters (Millipore Filter Corp., Bedford, Mass.) were attached with Barge Cement (Barge Cement Mfg. Co., Towaco, N. J.) Osmotic shock of phage could be carried out in these cups through the pipetting hole. This hole was covered firmly with tape after the contents were added.

Extraction and Dialysis of Chromosomes

Phage chromosomes were released by osmotic shock from 2.5 to 2.6 M sucrose (Brenner and Barnett, 1959) or by dialysis for 20 min at room temperature against 5 M $NaClO_4$, 0.005 M EDTA, 0.05 M NaCl at pH 8.0 (Freifelder, 1966). Bacterial chromosomes were released by lysozyme (experiment 1) or sodium lauryl sulfate treatment (experiments 1 and 2) by a procedure similar to the one of Cairns (1963 *a*, *b*) except for the omission of carrier DNA and, in experiment 1, omission of NaCl. After release, the chromosomes were dialysed against 0.005 M EDTA, pH 8.0 at 37°C in experiment 1. In experiment 2, dialysis was against 0.005 M EDTA, 0.05 M NaCl, pH 8.0 at room temperature (except that when detergent was used, initial dialysis was at 37°C). Dialysis was for 12–18 hr. Dialysis baths were changed every 2 hr. In experiment 2, some fully processed detergent-released bacterial chromosomes were dialysed against the $NaClO_4$ solution as used for phage and subsequently against two changes of the EDTA dialysis solution.

After dialysis, chromosomes were collected on the Millipore filters by draining the cups as described by Cairns (1962).

Radioautography

The Millipore filters were glued to gelatin-coated slides and covered with Kodak AR-10 stripping film (Eastman Kodak Co., Rochester, N. Y.). Storage for exposure was at 18°C (Caro, 1964) over $CaSO_4$. Time of exposure was about 50 days in experiment 1 and 60 days in experiment 2. Development was for 20 min in complete darkness in Kodak developer D-19b at 19–20°C.

Selection of Chromosomes and Grain Counting

Chromosome pieces to be photographed were chosen as the longest and straightest ones available. The entire length of a chromosome piece was always photographed in successive views in bright-field illumination at 400× magnification, using Kodak Plus-X Pan film. Printing was on Kodabromide F-3 at a final magnification of 3570×.

The grains produced by a chromosome and all nearby background grains were traced onto tracing paper. The tracing of the chromosome was divided into segments 1 cm long, each section being equivalent to a chromosomal length of 2.8 μ. Grains were considered to be of chromosomal origin if they occurred within a 1 cm wide path centered on the chromosome. For each 1 cm^2 chromosome segment, the background grains in each of the square centimeters immediately to the right and left were recorded.

Three ways of estimating grain density per unit length for each kind of chromosome were used in experiment 1 and four ways in experiment 2. First, in both experiments, the number of grains associated with the chromosome, minus half the sum of the right-hand and left-hand background grains, divided by the length in microns gave grains per micron for each chromosome piece photographed. The grains per micron of all chromosomes of the same type, weighted by length, could then be averaged.

Second, to avoid possible bias due to short, tangled regions with high counts or small breaks giving low counts, modes were obtained from column diagrams made for each type of chromosome (see Figs. 4 *a* and 4 *b*). The grains in each sequential 2.8 μ length, minus half the associated right-hand and left-hand background grains, were recorded in experiment 1. In experiment 2 a unit length of 5.6 μ was used.

Third, in order to avoid statistical fluctuation effects in obtaining the modes of grains per 5.6 μ, independent modes were also obtained for sequential 8.4-μ lengths in experiment 2.

Fourth, weighted numerical modes were obtained from all of the column diagrams by use of the formula (Croxton, 1959),

$$M = L + \frac{D_1}{D_1 + D_2} i,$$

where M is the weighted mode, L is the lower limit of the modal class, D_1 is the difference between the frequency of the class to the left of the modal class (graphically) and the frequency of the modal class, D_2 is the difference between the frequency of the class to the right of the modal class and the frequency of the modal class, and i is the standard unit size of each class.

RESULTS

Growth of Bacteria and Phage in Labeled Medium

When *E. coli* B3 was grown in F medium supplemented with tritiated thymidine, growth did not conform to the usual pattern of exponential increase followed by stationary phase. Instead, increase in cell number was consistent with exponential growth for two or three generations, after which size increased with formation of chains or snakes, and this was followed by 14 hr of no apparent growth. Subsequently, a population of single cells was seen which appeared to grow exponentially. No change in the amount of material in chains was observed. Stationary phase for the "second growth" single cells occurs at about 8×10^8 cells/ml. The chains and the subsequently formed single cells were still thymine requiring. No thymine-independent revertants were found in stationary phase among 200 cells sampled in experiment 1 or 10^6 cells in experiment 2. *E. coli* B3 has a reversion rate which can be estimated as 1 in 10^{10} in cells growing with just limiting thymidine on a plate. All or most of the bacteria in chains were apparently alive since the chains rapidly disappeared after dilution into nonradioactive medium in experiment 2.

In each experiment cells were considered to be fully labeled when they entered their second growth phase after the temporary 14 hr lag, and after cell number had increased 100-fold in labeled media. An aliquot of cells was then removed for extraction of fully labeled chromosomes. A second aliquot was removed, added to 100-fold excess of unlabeled media and allowed to undergo a five-fold increase in cell number. Half-labeled (and unlabeled) chromosomes were extracted from these cells. Phage at 10^5/ml were added to a third aliquot of fully labeled cells. When partial clearing of the tube occurred, chloroform was added. There were then

5×10^9 viable labeled phage per ml in experiment 1 and 9×10^9 per ml in experiment 2.

Uniformity and Extent of Labeling

The reversion frequency of the bacteria to thymine independence was low, as discussed above, and no revertants of bacteriophage T_4Boltd8 were found among 1.2×10^4 phage tested by the plaque-size assay of Simon and Tessman (1963). Thus there should not have been any significant production of unlabeled thymidine by revertants. In all cases reproduction of the labeled organism was at least 100-fold in labeled medium, so that the contribution of unlabeled thymidine from the DNA of the parental inoculum was insignificant. The inactivation of labeled phage by tritium decays uniformly followed a single hit inactivation curve over the measured range of 100–0.05% survival which indicates uniformity of labeling. The tritium decays seemed to be more efficient in causing lethal damage under the conditions used here, where labeled phage were kept in Hershey broth at room temperature, than previously reported. The inactivation curve can be represented by the equation $N = N_0 e^{-kt}$, where k = lethals per phage per day, N = number of phage surviving at time t, and N_0 = initial number of phage at time zero. Here, for a specific activity of the tritiated thymidine of 12.4 Ci/mmole, k was 1.5. With this specific activity the value of k in the experiments of Cairns (1961) would have been 1.02 and k in the experiments of Caro (1965) would have been 1.08.

There is a small amount of activity in the defective thymidylate synthetase of *E. coli* B3. It has <1 to 2.5% of wild type activity in vitro (Barner and Cohen, 1959; Mathews, 1965) and allows a 1.6-fold increase in DNA content in 270 min of incubation at 37°C (Gallant and Suskind, 1961). There is apparently no significant activity remaining in the defective thymidylate synthetase of bacteriophage T_4Boltd8 (Mathews, 1965). After infection of *E. coli* B3 with T_4Boltd8 there is "reasonably good correspondence between the number of phage units per cell of thymine released from bacterial DNA and synthesis of phage DNA in the absence of thymine" (Mathews, 1966). Thus, under the conditions of Gallant and Suskind of a long period of incubation of *E. coli* B3 without thymine some DNA production can be seen, but under conditions of more normal rapid growth of T_4Boltd8 in *E. coli* B3 as performed by Mathews there was no indication of production of new intracellular thymine. Under the conditions of growth in the experiments reported here, the cold thymidine synthesized by the defective thymidylate synthetase may have contributed a few per cent of the total thymidine but it should not have significantly affected the relative specific activities of the thymidine in the *E. coli* and T_4 chromosomes.

Length of the T_4 Chromosomes

It is necessary to determine an average length for the T_4 chromosomes whose grain densities were obtained, in order to have a basis for estimating the absolute mass

per micron of these chromosomes from the weight values given by other workers. The lengths of 21 T_4Boltd8 DNA molecules measured in experiment 1 and 60 chromosomes measured in experiment 2 are given in Fig. 1. In experiment 1 not many untangled isolated molecules were obtained and, further, all molecules had been either poured or pipetted, so that most were broken. From the distribution of lengths

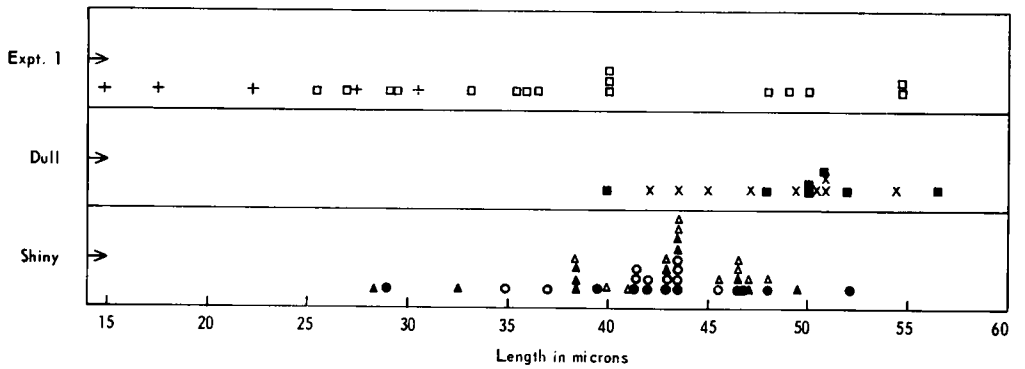

FIGURE 1 Lengths of radioautographic images of T_4 chromosomes. The lengths in the upper third of the figure were obtained in experiment 1. They were obtained from chromosomes deposited on the shiny sides of Millipore filters. □, molecules from a sample that was poured; +, molecules from a pipetted sample. The chromosome lengths given in the middle and in the lower third of the figure were obtained in experiment 2. Those in the middle were of chromosomes deposited on the dull sides of Millipore filters, and those in the lower third were of chromosomes deposited on the shiny sides of Millipore filters. The symbols denote the different slides on which the chromosome images were photographed.

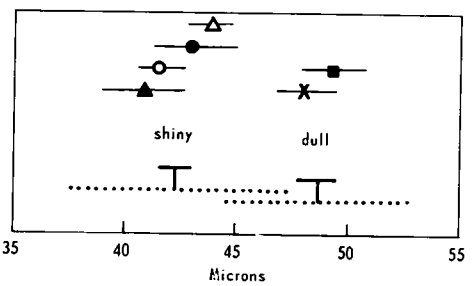

FIGURE 2 Chromosome lengths in experiment 2. In the lower half of the figure the mean length of all the phage chromosomes deposited on the shiny sides of Millipore filters and the mean length of all the phage chromosomes deposited on the dull sides are given by the short vertical lines. The words "shiny" and "dull" indicate the location of the respective means. The attached, dotted, horizontal lines represent the standard deviations of the samples. The attached, solid, horizontal lines are the standard errors of the mean.

In the upper half of the figure the mean lengths of the phage chromosomes on each of the six slides used are given by the six symbols. (The same symbols are used as in Figure 1.) The four slides on which chromosomes were deposited on the shiny sides of Millipores are above the word "shiny." The two slides where chromosomes are on the dull sides are above the word "dull." The standard errors of each of the means are given by the attached horizontal lines. The chromosomes represented by ×, ●, and ■ were extracted by osmotic shock. Those indicated by ○, △, and ▲ were extracted by $NaClO_4$.

it is probable that only the five longest molecules of average length 51.2 ± 1.5 μ are unbroken chromosomes. In experiment 1 only the shiny sides of Millipore membranes were used.

In experiment 2, where chromosomes were released within the dialysis cups without transfer, between 2 and 50% of all phage chromosomes on a membrane were unbroken. Here chromosomes for analysis were chosen as being relatively long and straight within an area of generally unbroken chromosomes. Of 18 chromosomes deposited on the dull sides of two Millipore membranes the average length was 48.7 ± 4.1 μ and of 42 on the shiny sides of three membranes it was 42.3 ± 4.8 μ. As shown in Fig. 1, the distributions of lengths on the two kinds of Millipore surfaces are overlapping. However (Fig. 2) the mean lengths on the two surfaces are significantly different. Thus it is likely that the lengths of the chromosomes are determined in part by their interaction with the surface on which they are dried. The method of extraction does not seem to make a difference (Fig. 2).

Relative Grain Densities of the Chromosomes

Typical radioautographs from which grain counts were made are shown in Fig. 3. Grains per unit length for the 12,636 μ of chromosomes analysed are compiled in Figs. 4 *a* and 4 *b*. In Fig. 4 *a* it can be seen that under the conditions of experiment 1 the combined data from molecules on two slides of fully labeled *E. coli* chromosomes give essentially the same grain density as the combined counts for two slides of phage chromosomes. Fig. 4 *b* presents the compiled data of five slides of fully labeled *E. coli* chromosomes, five slides of half-labeled *E. coli* chromosomes and six slides of phage chromosomes produced in experiment 2. It shows that in this experiment fully labeled *E. coli* chromosomes have somewhat less than twice the grain density of half-labeled *E. coli* chromosomes and that (in contrast to experiment 1) the fully labeled *E. coli* chromosomes produce a smaller grain density than phage T_1 chromosomes.

The weighted modes of the grains per micron for chromosomes in experiment 1, the means, modes, and weighted modes of grains per micron for chromosomes in experiment 2, and the means of grains per micron for chromosomes in Cairns' experiments (Cairns 1962, 1963 *b*) are shown in Table I. This table shows that the means in experiment 2 were 2–7% above the values of the averages of the weighted modes. This could result from a very small amount of kinking or just tailing of the curve of grains per micron on the high side, which is prevented on the low side by closeness of the curve to 0 grains/μ. The different weighted modes within a single class of DNA differ by 0–5% which provides an estimate of the statistical fluctuation in the values. The values of experiment 1 are not given in such detail because they were obtained from fewer pieces of film, and as discussed below, this would make them somewhat less reliable.

In evaluating the relationships between the grain densities of different kinds of

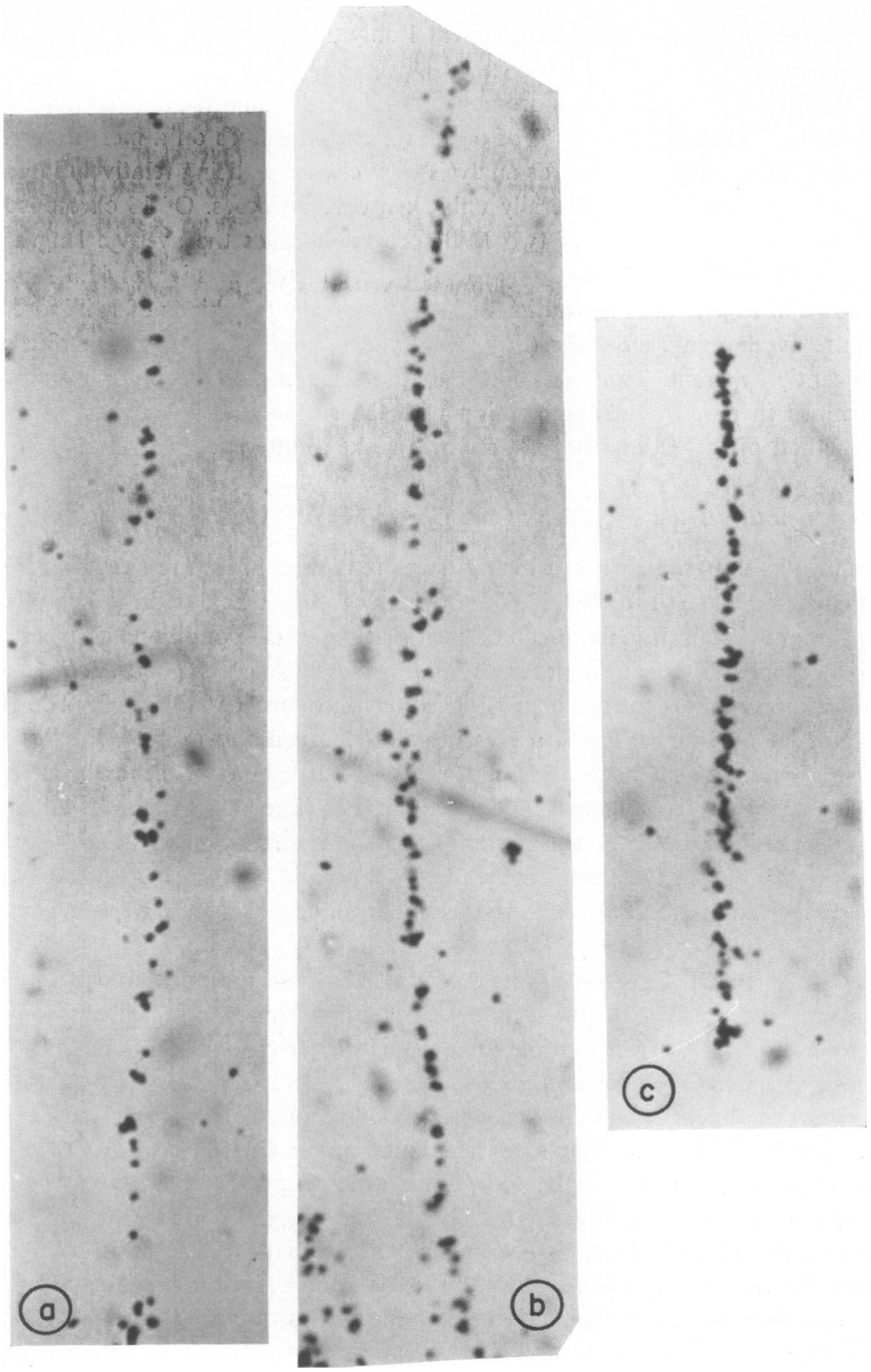

FIGURE 3 Typical photographs from which grain counts were made. The chromosome segments shown, and their lengths and grain densities are: (*a*) half-labeled *E. coli*, 76 μ, 0.85 grains/μ; (*b*) fully labeled *E. coli*, 85 μ, 1.23 grains/μ; (*c*) phage T_1, 47 μ, 1.56 grains/μ.

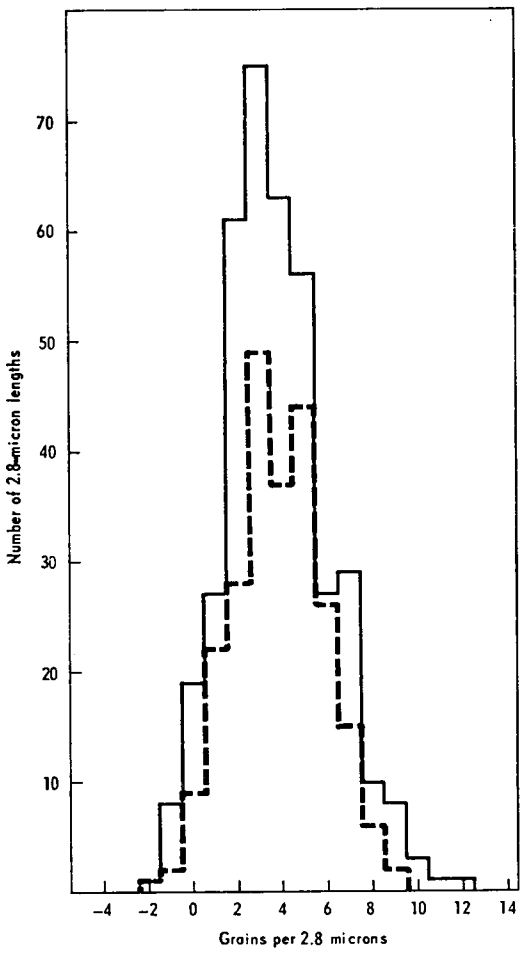

FIGURE 4 *a*

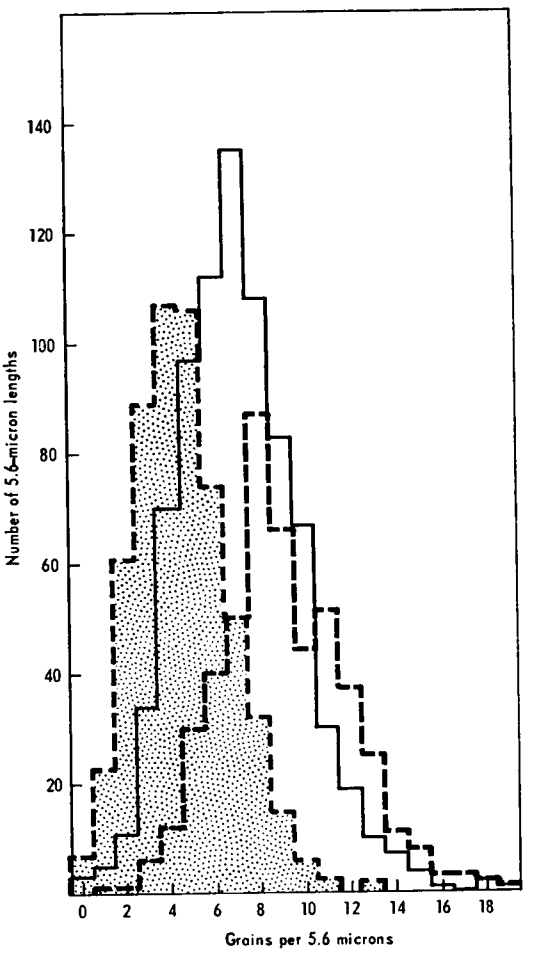

FIGURE 4 *b*

FIGURE 4 *a* Column diagrams of grain densities found in experiment 1. The number of radioautographic grains found in 2.8-μ lengths is given for fully labeled *E. coli* chromosomes (solid line) and phage chromosomes (dashed line).

FIGURE 4 *b* Column diagrams of grain densities found in experiment 2. The number of radioautographic grains found in 5.6-μ lengths is given for fully labeled *E. coli* chromosomes (solid line), half-labeled *E. coli* chromosomes (shaded area enclosed by dashed line), and phage chromosomes (dashed line).

chromosomes, a number of sources of random and systematic biases must be taken into account. The largest source of systematic bias, which is important in interpreting Table I, was the crowding of grains in the radioautographs of fully labeled *E. coli* chromosomes and phage chromosomes which led to undercounting. Thus, the fully labeled *E. coli* chromosomes of experiment 2 have an average weighted mode grain count of 1.23 grains/μ compared to 0.80 grains/μ for half-labeled

TABLE I

GRAINS PER MICRON* AND NUMBER OF MICRONS ANALYSED‡

	Mean (expt. 2)	Mode from grains per 5.6 μ (expt. 2, Fig. 4 *b*)	Weighted mode from grains per 5.6 μ (expt. 2, Fig. 4 *b*)	Mode from grains per 8.4 μ (expt. 2)	Weighted mode from grains per 8.4 μ (expt. 2)	Weighted mode from grains per 2.8 μ (expt. 1, Fig. 4 *a*)	Mean from Cairns, 1962, 1963 *b*
Fully labeled *E. coli* chromosomes	1.25 (4,475)	1.25 (4,475)	1.24 (4,475)	1.19 (4,475)	1.21 (4,475)	1.09 (1,096)	1.85 (885)
Half-labeled *E. coli* chromosomes	0.82 (3,367)	0.71 (3,367)	0.80 (3,367)	0.83 (3,367)	0.80 (3,367)	—	1.05 (885)
Phage chromosomes	1.60 (3,054)	1.43 (3,054)	1.45 (3,054)	1.55 (3,054)	1.52 (3,054)	1.12 (644)	2.4 (1,552)

* Note that grain density of a given type of chromosome will vary in different experiments because of differences in exposure of the radioautographs.

‡ First number of each pair — grains per micron; numbers in parentheses — number of microns.

E. coli chromosomes. The half-labeled chromosomes have an apparent grain count value which is 65% as large as that of the fully labeled chromosomes, rather than the expected 50%. Difficulty in counting grains in a chromosome became apparent when there was an average of more than 1 grain/μ.

The largest source of random variability was the heterogeneity of the sensitivity of the AR-10 stripping film as used here. This heterogeneity was probably due to differences in drying or other handling. The average lengths of the phage chromosomes were about the same on the same sides of Millipores (Fig. 2), so that the average numbers of nucleotides per micron were about the same. However, the mean grain densities per slide, where different pieces of film were used, were 4.41, 4.42, 4.46, and 5.47 grains/2.8 μ for phage chromosomes on the shiny sides of Millipore filters and 3.47 and 4.90 on the dull sides (Fig. 5). The standard deviations of the samples are 11 and 24% of their means, respectively.

It is not clear how much heterogeneity in sensitivity was present in experiment 1. Although the modes of the two *E. coli* slides were the same, 1.1 grains/μ measured on a unit distance of 2.8 μ, the means were 1.1 and 1.4 grains/μ. In view of the heterogeneity found in experiment 2, the small number of slides used in experiment 1 makes the data obtained from them subject to bias. Because of the lesser reliability of the values of experiment 1, they were not subject to the analysis performed on the values of experiment 2.

Another factor to be taken into account is the possibility that *E. coli* DNA, like phage DNA (see Fig. 2), assumes a more stretched-out conformation on the dull, compared to the shiny, side of Millipore filters. From the phage lengths the expected ratio of grain densities is dull/shiny = 0.87. The actual ratios obtained were half-labeled = 0.85, fully labeled = 0.97, and phage = 0.89. However, there was much variability and for each type of DNA only two dull and three shiny surfaces were

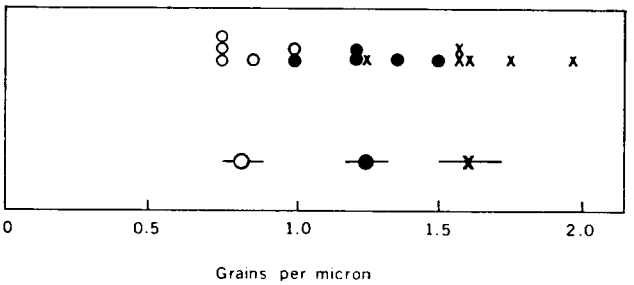

FIGURE 5 Average grains per micron in experiment 2. The upper half of the figure shows the average grains per micron for each slide of fully labeled *E. coli* chromosomes (●), half-labeled *E. coli* chromosomes (○), and phage chromosomes (×). The lower half of the figure gives the mean values for each type of slide and the error bars represent the standard errors of the means. The same symbols are used as in the upper half of the figure.

used for comparison, except for the phage chromosomes where two dull and four shiny surfaces were used. Thus, not enough data were collected to be sure bacterial chromosomes have different configurations on the two surfaces. The data from the two surfaces were treated separately in Table III, but for most calculations they were pooled (Figs. 4 *b* and 5, and Table I). Since data from similar proportions of dull and shiny Millipore surfaces made up the pooled data for each type of chromosome, error from this source is small when comparing grain densities.

The amount which wiggling, undetected by radioautography, may add to grain per micron estimates is probably not too great. The lengths obtained for T_4 chromosomes are similar to lengths obtained by electron microscope. The wiggles that can be seen in some electron micrographs of homogeneous undenatured DNA (see e.g. Beer, 1961) which can be expected to be missed in an radioautograph would contribute only an extra 3% to the length obtained without following these small wiggles.

Relative Numbers of Nucleotides per Unit Length

In experiment 1 the best estimate which can be made from the data is that fully labeled *E. coli* chromosomes and phage chromosomes produced about the same grain densities in radioautographs. Using molar proportions, *E. coli* DNA contains about 25% thymidine (see e.g. review of Belozersky and Spirin, 1960) and T_4 DNA contains about 33% thymidine (Wyatt and Cohen, 1953). For the two types of chromosomes to produce nearly equal grain counts in experiment 1, the *E. coli* chromosome would have had to have 1.3-fold more nucleotides per unit length than the T_4 chromosome.

Fig. 5 shows the mean grain densities obtained on each of the 16 slides analysed in experiment 2. Also shown are the means of these values and the standard errors of the means for each type of chromosome. Here, fully labeled *E. coli* chromosomes produced about 80% as many grains per micron as the T_4 chromosomes (see also

Fig. 4 *b* and Table I). As pointed out above, the *E. coli* DNA contains about 25% thymidine and the T_4 DNA contains about 33% thymidine. Taking these molar proportions of thymidine into account, the grain counts in experiment 2 imply that *E. coli* chromosomes have 1.06-fold the number of nucleotides per unit length of the T_4 chromosomes.

The difference in relative nucleotides per unit length for *E. coli* vs. phage T_4 in experiments 1 and 2 (1.3 and 1.06, respectively) is probably due to the smaller number of slides used in experiment 1 as was discussed above. The fairly close agreement in nucleotide densities in each experiment implies that *E. coli* and phage T_4 chromosomes have closely similar structures.

Semiconservative Replication of the E. coli Chromosome

Meselson and Stahl (1958) showed that a DNA-containing structure in *E. coli* was semiconserved during replication. They were careful to point out (Meselson and Stahl, 1958, 1966) that their experiments did not define the nature of the conserved subunit. Baldwin and Shooter (1963) provided data indicating the conserved unit was a single strand, but their data did not indicate how the DNA was arranged in a chromosome.

It has remained a possibility that the *E. coli* chromosome consists of a pair of Watson-Crick double helices as proposed by Cavalieri and coworkers (Cavalieri and Rosenberg, 1961 *a*, *b*, *c*; Cavalieri, Finston, and Rosenberg, 1961; Hall and Cavalieri, 1961). It could be proposed that each of the double helices replicate semiconservatively (Watson and Crick, 1953; Baldwin and Shooter, 1963) and the four helices produced assort randomly in pairs to produce new chromosomes. Under this scheme of replication, cells grown for many generations in labeled medium and then shifted to unlabeled medium for two or more generations would produce chromosomes of three types: half-labeled, quarter-labeled, and unlabeled. However, cells grown here in this manner were not observed to produce quarter-labeled chromosomes. The background was low enough so they would have been detectable, and coincidence of grains at the exposure used would be low so that quarter-labeled and half-labeled structures would have been easily distinguishable.

The only labeled chromosomes found after somewhat more than two generations growth in unlabeled medium were half-labeled (Figs. 3 and 4 *b*). Thus there are only two separable conserved units in the exponential phase *E. coli* chromosome. If the exponential phase chromosome consists of a pair of double helices either each double helix would be conserved during replication or else two of the four strands present would always segregate together during replication.

Cairns (1963 *a*), also had concluded that the *E. coli* chromosome had two subunits since he demonstrated the presence of a half-labeled structure. He also suggested that comparisons of grain counts between half-labeled *E. coli* chromosomes and fully labeled T_2 and λ chromosomes ruled out the possibility that *E. coli* chro-

mosomes could contain more than two strands in a Watson-Crick helix. However, this conclusion should be regarded as having been a tentative one since grain counts were only given for a single *E. coli* chromosome and grain counting for the phage chromosomes was done in a separate experiment where sensitivity of the radioautographic film may have been different due to differences in age of the film or developer. The present experiments indicate that if the exponential phase *E. coli* chromosome has four strands they do not segregate at random. This interpretation could not have been obtained from the data Cairns presented (Cairns, 1963 *a*, *b*, 1966). In his experiments three-quarter-labeled chromosomes could have existed but have been confused with fully labeled chromosomes unless grain counts were made. At the high grain density he used for fully labeled chromosomes, about 1.85 grains/μ (Cairns, 1963 *b*), it is often not easy to assess grain density by eye. Quarter-labeled chromosomes would not have been expected since his cells were initially grown in unlabeled medium, transferred to labeled medium, and sampled after two generations.

Constraints on Chromosome Unfolding

The 1100–1400 μ long, intact, replicating, *E. coli* chromosome (Cairns, 1963 *a*, *b*, 1966) should contain two fork regions (Cairns 1963 *b*, 1966). Of the 150 to 400-μ length fragments usually found untangled, only about 2–5% had fork regions. Thus fork regions are probably preferentially not displayed. (As expected, no forks were observed among half-labeled chromosomes.)

It is thought (Hershey, 1953) that about 50 phage equivalents of DNA produced during infection are not incorporated into mature phage heads. It is interesting that this nonmature DNA was not displayed in extended form when 10 samples of the supernatant of a centrifuged bacterial lysate containing intact phage were dialysed and radioautographs were made of the Millipore membranes. This nonmature DNA may be constrained from unfolding even under conditions of prolonged dialysis.

DISCUSSION

Configuration of T_4 and E. coli DNA

In determining the configuration of T_4 and *E. coli* DNA the number of nucleotides per 3.4 A must be calculated. For T_4 DNA this can be done using the following equation:

$$N_{3.4} = \frac{M_D}{M_N} \times \frac{3.4 \text{ A}}{L},$$

where $N_{3.4}$ = the number of nucleotides per 3.4 A, M_D = a molecular weight estimate for the DNA of phage T_4 or the related T_2 (these are summarized in Table II), M_N = the average molecular weight of a nucleotide in T_4 or T_2 DNA (these

TABLE II

MOLECULAR WEIGHT ESTIMATES IN DALTONS $\times 10^{-6}$ OF T_2 AND T_4 DNA MOLECULES AND THE CORRESPONDING NUMBER OF NUCLEOTIDES PER 3.4 A CALCULATED FOR T_4 CHROMOSOMES OF LENGTH 42.8 μ*

Molecular weight	Method of determination	Year	Organism and reference§	Nucleotides per 3.4 A
91	Determination of per cent P and of mol. wt. of phage	1960	T_2 (1)	2.18
96‡	Sedimentation coefficient and viscosity	1965	T_4 (2)	2.29
99‡	Sedimentation equilibrium	1969	T_4 (3)	2.32
113‡	Sedimentation coefficient	1964	T_4 (4)	2.70
122	Determination of per cent P	1952	T_2 (5)	2.91
125‡	P^{32} radioautography	1961, 1969	T_2 (6, 7)	2.98
126‡	Sedimentation coefficient of single strand	1965	T_2 (8)	3.01
130	Determination of micrograms P per plaque-forming unit	1953	T_2 (9)	3.10
151	Determination of number of P atoms per phage	1955	T_2 (10)	3.60
152‡	Optical cross-section at 260 mμ and determination of micrograms P per plaque-forming unit.	1961	T_2 (6)	3.62

* The mean length of 42.8 μ was obtained for T_4 chromosomes on the shiny side of Millipore filters in experiment 2 (Fig. 2). Note, however, that under other conditions the T_2 and T_4 chromosomes assume longer lengths. The lengths of 48.7 μ (Fig. 2, this work), 52.3 μ (Cairns, 1961), 55.0 μ (Thomas, 1966), and 55.5 μ (Thomas and MacHattie, 1964) would yield values of nucleotides per 3.4 A which are 88%, 82%, 78%, and 77%, respectively of the values listed.

‡ These values have been calculated from the values given for the sodium salts of the DNA's. The molecular weights of T_2 and T_4 DNA's are 94% of their sodium salts.

§ Numbers below in parentheses refer to the following: (1) Cummings and Kozloff, 1960; (2) Aten and Cohen, 1965; (3) Schmid and Hearst, 1969; (4) Roller, 1964; (5) Herriot and Barlow, 1952; (6) Rubenstein, Thomas, and Hershey, 1961; (7) Leighton and Rubenstein, 1969; (8) Studier, 1965; (9) Hershey, Dixon, and Chase, 1953; (10) Stent and Fuerst, 1955.

are taken as 342 Daltons for T_4 and 336.5 Daltons for T_2 ; the values were calculated using the per cent HMC found by Wyatt and Cohen [1953] and the per cent glucosylation given in Lehman and Pratt [1960]), and L = the length of the T_4 chromosome in angstrom units.

For T_4 DNA with a length of 42.3 μ, as obtained in experiment 2 on the shiny sides of Millipore filters, the values of $N_{3.4}$ corresponding to the 10 independent values of M_D which occur in the literature are given in Table II. (Values of M_D which depend on assuming a B configuration for T_4 DNA are not included.)

The number of nucleotides per 3.4 A in the *E. coli* chromosome can be calculated by comparing its mean grain density with that of the T_4 chromosome, and adjusting for the different molar proportions of thymidine in the two chromosomes (25% thymidine in *E. coli* DNA and 33% thymidine in T_4 DNA). The equation used

was:

$$N_{3.4E} = \frac{(0.33)G_E}{(0.25)G_T} N_{3.4T},$$

where $N_{3.4E}$ = the number of nucleotides per 3.4 A in the *E. coli* chromosome, $N_{3.4T}$ = the number of nucleotides per 3.4 A in the T_4 chromosome, G_E = the grain density produced by the *E. coli* chromosome, and G_T = the grain density produced by the T_4 chromosome.

The values of $N_{3.4E}$ and $N_{3.4T}$ were calculated for the three different experimental conditions used in experiments 1 and 2 and using the highest, lowest, and median molecular weight estimates for the T_4 chromosome. These values are given in Table III. The extreme values for the *E. coli* chromosome were 2.10 and 3.89 nucleotides/3.4 A and for the T_4 chromosome were 1.80 and 3.62 nucleotides/3.4 A. By comparison, the B form of the Watson-Crick DNA helix has 2.0 nucleotides/3.4 A.

Table III shows that the calculated values for the number of nucleotides per 3.4 A in the *E. coli* and T_4 chromosomes on the same surfaces are very close in experiment 2, where the most data was obtained, and are not too far apart in preliminary experiment 1. Within the accuracy of the measurement one would say the *E. coli* chromosome has about the same number or slightly more nucleotides per 3.4 A than the T_4 chromosome. This implies that the chromosomes of both organisms are closely similar or identical in their secondary molecular configuration.

It would be very attractive to be able to determine the strandedness of these chromosomes by the estimates obtained here of their number of nucleotides per 3.4 A.

TABLE III

NUCLEOTIDES PER 3.4 A FOR T_4 AND *E. coli* CHROMOSOMES UNDER VARIOUS CONDITIONS ASSUMING MOLECULAR WEIGHTS OF T_4 CHROMOSOMES AS 91×10^6, 122×10^6, OR 152×10^6 DALTONS*

Chromosome	Exp't.	Side of membrane	Length in microns	Nucleotides per 3.4 A for mol. wt.		
				91×10^6	122×10^6	152×10^6
T_4	2	Dull	48.7	1.89	2.53	3.14
E. coli	2	Dull	—	2.10	2.81	3.48
T_4	2	Shiny	42.3	2.18	2.91	3.62
E. coli	2	Shiny	—	2.22	2.97	3.69
T_4	1	Shiny	51.2	1.80‡	2.40‡	2.99‡
E. coli	1	Shiny	—	2.34‡	3.12‡	3.89‡

* The molecular weights of the T_4 chromosome chosen for this table are the two extreme values and the average value of all the weights given in Table II.

‡ Values obtained in experiment 1 should be given less weight than those of experiment 2 since less data was obtained in experiment 1.

Unfortunately these values could be consistent with them being either one Watson-Crick helix approximately in the B form or more condensed than the B form or two helices less condensed than the B form. Both more condensed forms, the crystalline A form (Fuller, Wilkins, Wilson, and Hamilton, 1965) and C form (Marvin, Spencer, Wilkins, and Hamilton, 1961), and a less condensed Cs-salt form (Luzzati, 1963) are known to exist.

On Choosing a Probable Configuration

It has been shown here that the *E. coli* chromosome has a number of nucleotides per unit length just about equal to that of the T, chromosome. Thus the two kinds of chromosomes probably have the same strandedness and configuration.

Although it is generally accepted that the T_4 chromosome is a two-stranded Watson-Crick double helix, the evidence is not entirely conclusive on this point. The finding of Beer and Zobel (1961) that the height and width of the related T_2 chromosome was 20–25 A would be consistent with the T_2 chromosome being a Watson-Crick helix, but would also be consistent with the four-stranded DNA structure described by Wu (1968, 1969). In the work reported by Cavalieri and Rosenberg (1961 *a*) the T_2 chromosome did not halve in molecular weight upon heating to 100°C in CsCl. This was a characteristic shared by chromosomes which behaved as two-stranded with respect to DNase and X-ray scission kinetics. However, this evidence is fairly indirect. Finally, if the molecular weight estimates for T_2 and T_4 chromosomes which are under 100×10^6 Daltons are most nearly correct, then the longer lengths which the T_2 and T_4 chromosomes can attain, 51.2 μ (this work), 52.3 μ (Cairns, 1961), 55.0 μ (Thomas, 1966), and 55.5 μ (Thomas and MacHattie, 1964) would require a structure with less than 2 nucleotides/3.4 A and this would be incompatible with a four-stranded structure even though completely stretched out (Luzzati, Mathis, Masson, and Witz, 1964). The other molecular weight estimates would not be incompatible with a four-stranded structure.

Experiments on the strandedness of the *E. coli* chromosome have been much more direct but have led to conflicting results.

Cavalieri and Rosenberg (1961 *a*, *b*, *c*) have presented DNase scission kinetic data indicating that exponential phase *E. coli* chromosomes contain a pair of double helices while chromosomes from *E. coli* in an artificial stationary phase contain one double helix. Luzzati, Luzzati, and Masson (1962) reported that *E. coli* DNA's extracted from an artificial stationary phase and from exponential phase, which reacted in solution as two-stranded and four-stranded, respectively, by the tests of Cavalieri (1961 *c*), have the same local mass per unit length in solution as determined by low angle X-ray scattering. Luzzati and coworkers (1962, 1963) are careful to point out that their measurements would not distinguish between a single Watson-Crick helix and a pair of double helices joined at distances greater than every 300 A (about nine turns of a B form helix).

On the other hand, Baldwin and Shooter (1963) have shown that a hybrid *E. coli* chromosome, labeled in one conserved subunit with 5-BU, melted out in a single step from native to denatured form as the pH was raised. They argue that if an intact helix were conserved during replication, so that both a labeled and an unlabeled helix were present in the hybrid chromosome, the melting out should occur in two steps. Thus their data indicate that the conserved subunit is a single strand of a double helix. In conjunction with the experiments reported here which indicate that the exponential phase *E. coli* chromosome contains only two conserved subunits, these experiments imply that the exponential phase *E. coli* chromosome is only one helix.

If the widely accepted assumption that the T_4 chromosome is two-stranded is correct, then the present work, which indicates that the *E. coli* chromosome has a number of nucleotides per 3.4 A closely similar to the T_4 chromosome, would favor the conclusion that the *E. coli* chromosome is also two stranded.

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REFERENCES

ADAMS, M. D. 1959. Bacteriophages. Interscience Publishers, Inc. New York.
ATEN, J. B. T., and J. A. COHEN. 1965. *J. Mol. Biol.* **12**:537.
BALDWIN, R. L., and E. M. SHOOTER. 1963. *J. Mol. Biol.* **7**:511.
BARNER, H. D., and S. S. COHEN. 1959. *J. Biol. Chem.* **234**:3987.
BEER, M. 1961. *J. Mol. Biol.* **3**:263.
BEER, M., and C. R. ZOBEL. 1961. *J. Mol. Biol.* **3**:717.
BELOZERSKY, A. N., and A. S. SPIRIN. 1960. *In* The Nucleic Acids. Chargaff and J. N. Davidson, editors. Academic Press, Inc., New York. **3**:147–185.
BRENNER, S., and L. BARNETT. 1959. Structure and Function of Genetic Elements. Brookhaven Symposia in Biology, No. 12. Brookhaven National Laboratory, Upton, New York. 86–94.
CAIRNS, J. 1961. *J. Mol. Biol.* **3**:765.
CAIRNS, J. 1962. *Cold Spring Harbor Symp. Quant. Biol.* **27**:311.
CAIRNS, J. 1963 *a. J. Mol. Biol.* **6**:208.
CAIRNS, J. 1963 *b. Cold Spring Harbor Symp. Quant. Biol.* **28**:43.
CAIRNS, J. 1966. *Sci. Amer.* **214**:36.
CARO, L. G. 1964. Methods in Cell Physiology. D. M. Prescott, editor. Academic Press, Inc., New York. **1**:327–363.
CARO, L. G. 1965. *Virology.* **25**:226.
CAVALIERI, L. F., and B. H. ROSENBERG. 1961 *a. Biophys. J.* **1**:317.

CAVALIERI, L. F., and B. H. ROSENBERG. 1961 *b*. *Biophys. J.* **1**:323.
CAVALIERI, L. F., and B. H. ROSENBERG. 1961 *c*. *Biophys. J.* **1**:337.
CAVALIERI, L. F., R. FINSTON, and B. H. ROSENBERG. 1961. *Nature* (*London*). **189**:833.
CROXTON, F. E. 1959. Elementary statistics. Dover Publications, Inc., New York.
CUMMINGS, D. J., and L. M. KOZLOFF. 1960. *Biochim. Biophys. Acta*. **44**:445.
FREIFELDER, D. 1966. *Virology*. **28**:742.
FULLER, W., M. H. F. WILKINS, H. R. WILSON, and L. D. HAMILTON. 1965. *J. Mol. Biol.* **12**:60.
GALLANT, J., and S. R. SUSKIND. 1961. *J. Bacteriol.* **82**:187.
HALL, C. E., and L. F. CAVALIERI. 1961. *J. Biophys. Biochem. Cytol.* **10**:347.
HERRIOT, R. M., and J. F. BARLOW. 1952. *J. Gen. Physiol.* **36**:17.
HERSHEY, A. D. 1953. *J. Gen. Physiol.* **37**:1.
HERSHEY, A. D., J. DIXON, and M. CHASE. 1953. *J. Gen. Physiol.* **36**:777.
JOSSE, J., and J. EIGNER. 1966. *Annu. Rev. Biochem.* **35**:789.
KOZLOFF, L. M. 1953. *Cold Spring Harbor Symp. Quant. Biol.* **18**:209.
LEHMAN, I. R., and E. A. PRATT. 1960. *J. Biol. Chem.* **235**:3254.
LEIGHTON, S. B., and I. RUBENSTEIN. 1969. *J. Mol. Biol.* **46**:313.
LUZZATI, V. 1963. *In* Progress in Nucleic Acid Research. J. N. Davidson and W. E. Cohn, editors. Academic Press, Inc., New York. **1**:347–368.
LUZZATI, V., D. LUZZATI, and E. MASSON. 1962. *J. Mol. Biol.* **5**:375.
LUZZATI, V., A. MATHIS, F. MASSON, and J. WITZ. 1964. *J. Mol. Biol.* **10**:28.
MARVIN, D. A., M. SPENCER, M. H. F. WILKINS, and L. D. HAMILTON. 1961. *J. Mol. Biol.* **3**:547.
MATHEWS, C. K. 1965. *J. Bacteriol.* **90**:648.
MATHEWS, C. K. 1966. *Biochemistry*. **5**:2092.
MESELSON, M., and F. W. STAHL. 1958. *Proc. Nat. Acad. Sci. U.S.A.* **44**:671.
MESELSON, M., and F. W. STAHL. 1966. *In* Phage and the Origins of Molecular Biology. J. Cairns, G. S. Stent, and J. D. Watson, editors. Cold Spring Harbor Laboratory of Quantitative Biology, Cold Spring Harbor, New York. 246–251.
PEACOCK, W. J. 1963. *Proc. Nat. Acad. Sci. U.S.A.* **49**:793.
ROLLER, A. 1964. *J. Mol. Biol.* **9**:260.
RUBENSTEIN, I., C. A. THOMAS, JR., and A. D. HERSHEY. 1961. *Proc. Nat. Acad. Sci. U.S.A.* **47**:1113.
SCHMID, C. W., and J. E. HEARST. 1969. *J. Mol. Biol.* **44**:143.
SIMON, E. H., and I. TESSMAN. 1963. *Proc. Nat. Acad. Sci. U.S.A.* **50**:526.
STEINBERG, C. M., and R. S. EDGAR. 1962. *Genetics*. **47**:187.
STENT, G. S., and C. R. FUERST. 1955. *J. Gen. Physiol.* **38**:441.
STUDIER, F. W. 1965. *J. Mol. Biol.* **11**:373.
TAYLOR, J. H. 1963. *In* Molecular Genetics. J. H. Taylor, editor. Academic Press, Inc., New York. **1**:65–111.
THOMAS, C. A., JR. 1966. *J. Gen. Physiol.* **49**(6, Pt. 2):143.
THOMAS, C. A., and L. A. MACHATTIE. 1964. *J. Mol. Biol.* **52**:1297.
WAHLEN, K. H. 1965. *Genetics*. **51**:915.
WATSON, J. D., and F. H. C. CRICK. 1953. *Cold Spring Harbor Symp. Quant. Biol.* **18**:123.
WU, T. T. 1968. *Bull. Math. Biophysics*. **30**:681.
WU, T. T. 1969. *Proc. Nat. Acad. Sci. U.S.A.* **63**:400.
WYATT, G. R., and S. S. COHEN. 1953. *Biochem. J.* **55**:774.