Summary.- RNA polymerase, purified 500 fold from extracts of *Azotobacter vinelandii*, used polyribonucleotides (poly A, poly U, poly C) in addition to DNA as primers. Priming by homopolynucleotides directed the incorporation of the complementary nucleotides. There was competition between the various primers suggesting that a single enzyme is involved in the reactions studied. Poly fluorouridylate was an effective inhibitor of both the DNA- and polyribonucleotide-primed reactions but actinomycin D, which inhibits the reaction primed by DNA, had no appreciable effect on the polyribonucleotide-primed reaction.

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[6] The abbreviations used in this paper are: RNA and DNA, ribo- and deoxyribonucleic acid, respectively; TMV, tobacco mosaic virus; dAT, deoxyadenylate-thymidylate copolymer; ATP, GTP, UTP, and CTP, the 5′-triphosphates, and AMP, GMP, UMP, and CMP, the 5′-monophosphates, respectively, of adenosine (A), guanosine (G), uridine (U), and cytidine (C); ADP, adenosine 5′-diphosphate; dATP, deoxyadenosine 5′-triphosphate; Tris, tris(hydroxymethyl) aminomethane. In polynucleotide chains the capital letters A, G, U, C, T, I, and FU are used for the above nucleosides plus thymidine, inosine, and fluorouridine, respectively.

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*THE STRUCTURE OF THE DNA-ACRIDINE COMPLEX**

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It has been proposed that acridines and related compounds bind to DNA by intercalation between normally neighboring base pairs in a plane perpendicular to the helix axis (Lerman, 1961); the space is provided by an extension and local untwisting of the helix. To test this hypothesis further, some other properties of the

DNA-acridine complex have been examined in dilute aqueous solution, including the orientation of the plane of the acridine and the effect of complexing on the orientation of the plane of the purines and pyrimidines, both with respect to the DNA helix axis. The results show that the bound acridine is more nearly perpendicular than tangent to the helix axis, and the perpendicularity of the base pairs to the helix axis is not significantly altered. In a separate study of the effect of complexing on the rates of diazotization of amino acidines, and the effect of complexing on the reactivity of purine and pyrimidine amino groups, it is also shown that the amino groups of amino acridines are relatively inaccessible within the complex, and hydrogen bonding between base pairs remains undisturbed (Lerman, 1963).

Experimental Methods.—Polarized fluorescence was measured at 90° to the incident beam in a conventional instrument constructed in this Department of Biophysics. Monochromatic polarized light was obtained from a tungsten or deuterium arc source by means of a Beckman DU monochromator fitted with a rotatable calcite Glan prism. The sample was examined at 25.0°C in either a 1 cm square fused silica cuvette or a fused silica capillary tube. The intensity of the emission was determined through an appropriate long wave-length pass filter and a calcite Glan-Thompson prism by a chilled, low-noise (EMI 9536) photomultiplier, operated as a photon counter. Flow of the DNA solution was effected by a synchronous motor-driven syringe. All measurements represent roughly 10^5 counts or more (about 100 $\times$ background), accumulated in one or more ten-second periods. Flow dichroism was measured in a Cary Model 14 spectrophotometer, using the Glan prism. The solution was driven through a short-path cuvette with planar silica windows, separated by 0.0271 cm, perpendicular to the light beam.

All measurements were carried out with chicken erythrocyte DNA isolated from washed nuclei by phenol extraction, similar to the method of Kirby (1958).

Recognition of the Acridine Plane.—If an array of stationary, randomly oriented fluorescent molecules is illuminated with suitable exciting radiation, the emitted light is polarized; that is, the emitted intensity observed through a polarizing prism will depend on the orientation of the prism. Although polarized emission is also observed when the exciting radiation is unpolarized, the degree of polarization of the emitted light is less. All of the following discussion and experimental results will refer to polarized excitation. Where emission is observed at right angles to the exciting beam, it is appropriate to compare intensities found when the analyzing prism is oriented perpendicular and parallel to this plane. When the exciting beam enters along the X axis, and emission is observed along the Y axis, the degree of polarization is given by the difference between the two intensities measured with the plane of polarization of the analyzing prism respectively parallel to Z and to X, divided by the sum of the same intensities. The probabilities of absorption and of emission with a particular polarization are proportional to the square of the cosine of the angle between the electric vector of the absorbed or emitted light and the direction of charge displacement, the transition moment, corresponding to the electronic transition responsible for absorption or emission. It can be shown that the Z intensity is greater and, consequently, the degree of polarization is positive, when the electronic transition moments for absorption and emission are more or less parallel; when they are more nearly perpendicular, the X intensity will be larger and the degree of polarization will be negative (Pringsheim, 1949). Since we shall be concerned only with singlet $\pi - \pi^*$ transitions, the transition moments for both absorption and emission will lie in the plane of the aromatic rings. It has been found, almost without exception, that the spectral distribution of emission from solutions of fluorescent substances is independent of the wavelength of excitation. Thus, the variation of the degree of polarization of emission with the wavelength of excitation corresponds to changes in direction only of the transition moment for excitation. The actual value of the degree of polarization may be taken to reflect the distribution of excitation probabilities between two or more different transitions that can be excited by the same wavelength (Albrecht, 1960). If the fluorescent molecules are allowed to rotate instead of remaining stationary during the interval between absorption and emission, the observed polarization will either disappear or be diminished depending upon the extent of randomization. In sufficiently concentrated solution, the emission may also be depolarized by migration of the excitation to nearby similar molecules. Polarization spectra for dilute solutions of the acridine deriva-

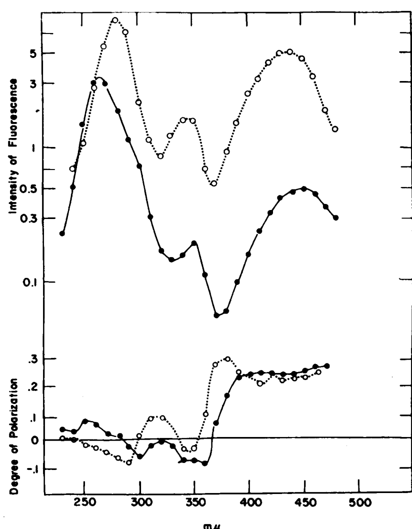

FIG. 1.—Excitation and polarization spectra for quinacrine in sucrose and DNA solution. The upper curves show the relative fluorescence intensities per unit concentration as a function of the wavelength of excitation, uncorrected for the energy distribution in the exciting radiation, for dilute solutions of quinacrine in DNA (solid line) and in 60% sucrose (dotted line). The lower curves show the degree of polarization of the 90° emission at each wavelength. The DNA concentration was about 3.6×10^{-4} *M* in nucleotides. The quinacrine was 2.65×10^{-6} *M* in the DNA solution, corresponding to one dye molecule per 68 nucleotide pairs. In the sucrose solution, the quinacrine was 1.51×10^{-7} *M*.

tive, quinacrine (Atebrin), are shown in Figure 1, together with the corresponding excitation spectra, uncorrected for the energy distribution of the exciting source. Although almost no polarization is observed in simple aqueous solutions, substantial polarization is observed in DNA solution because nearly all of the quinacrine is tightly bound, and DNA, by virtue of its molecular weight and configuration, is characterized by an exceedingly small rotational diffusion constant. (Similarly, the viscosity of a concentrated sucrose solution is sufficient to prevent a large rotational depolarization in the absence of DNA.) It will be noted that the long wave excitation maximum is shifted to somewhat longer wavelengths by attachment to DNA, as is found for the absorption maximum of proflavine (Peacocke and Skerrett, 1956) and other acridines (Morthland *et al.*, 1954). Although the fluorescence yield is generally much less for the bound than the free dye, a relatively increased emission probability can also be seen in the region of ultraviolet absorption by DNA. This presumably corresponds to sensitization of fluorescence and will be treated in a separate communication. From these data the wavelengths of 450 mμ and 300 mμ were selected as providing adequate fluorescence intensities with parallel (||) and perpendicular (⊥) pairs of transition moments. The directions of these transitions serve to specify the plane of the molecule.

The Effect of Flow.—It will be useful to consider a simplified scheme in which the transition moments for absorption and emission must lie either parallel or perpendicular to the flow axis, and the DNA molecule is assumed to be fully oriented with the helix axis parallel to the flow axis, and randomly rotated around the flow axis. Let us also restrict our attention to just two pairs of prism orientations—both prisms vertical, parallel to flow, and both horizontal, perpendicular to flow.

There are then three ways in which an acridine might be attached to the DNA helix, as indicated by types TL, TS, and P in Table 1 and Figure 2, and a set of four experimental predictions for each. The experimental measurement consists in the comparison of the fluorescence intensities of the flowing and stationary solution, given simply by the relative difference in intensities obtained when the DNA is oriented by flow and when its orientation is random. If a particular transition is that rendered parallel to the relevant prism orientation, the probability of observing transition will increase from the average value for random orientation to the maximum value; if the transition is rendered perpendicular to the prism orientation, it will not be observed at all. It will be seen that where one transition for either absorption or emission is parallel to a prism and the other is perpendicular, the product of the absorption and emission probabilities nevertheless equals zero.

TABLE 1

THE EXPECTED EFFECTS OF FLOW ORIENTATION ON POLARIZED TRANSITION PROBABILITIES ACCORDING TO THE BINDING ARRANGEMENT

	A. Orientation with respect to the helix axis			
1.	type of arrangement	TL	TS	P
2.	plane of dye	tangent	tangent	perpendicular
3.	emission transition	perpendicular	parallel	perpendicular
4.	‖ absorption transition	perpendicular	parallel	perpendicular
5.	⊥ absorption transition	parallel	perpendicular	perpendicular
	B. Difference in intensities, (flow oriented) − (random); both prisms parallel to flow			
6.	absorption only, ‖ transition	−	+	−
7.	absorption only, ⊥ transition	+	−	−
8.	emission only, assuming equal excitation	−	+	−
9.	emission from ‖ absorption	−	+	−
10.	emission from ⊥ absorption	−	−	−
	C. Difference in intensities, (flow oriented) − (random); both prisms perpendicular to flow			
11.	absorption only, ‖ transition	+	−	+
12.	absorption only, ⊥ transition	−	+	+
13.	emission only, assuming equal excitation	+	−	+
14.	emission from ‖ absorption	+	−	+
15.	emission from ⊥ absorption	−	−	+

The signs, + and −, signify that the flow oriented intensity is either greater or less, respectively, than the intensity for nonoriented molecules. If perfect orientation were achieved (infinitely great shear, etc.), all of the − values would approach zero. Lines 6, 7, 11, and 12 indicate changes that can, in principle, be observed as dichroism due to flow. Lines 9, 10, 14, and 15 indicate the net changes due to the product of the absorption and emission probabilities that would be expected in the fluorescence.

Since the functions are nonlinear, the effect of the decline will usually prevail when orientation is incomplete, and a net decrease in intensity will be observed.

Since the DNA molecule in solution appears to be a statistical coil of limited flexibility, only partial orientation can be expected, and the change in intensities induced by flow will be much less marked than the simplified theory predicts. A qualitative interpretation of the experimental results is possible, however, in terms of the direction of each intensity change induced by flow for each transition at each pair of prism orientations, together with an estimate of the degree of orientation of the DNA. Experimental results for quinacrine, presented in Table 2, should be compared with the expectations presented in lines 9, 10, 14, and 15 of Table 1 for the three hypothetical orientations. Since both intensities decrease during flow when the prisms are parallel to flow and both increase when the prisms are perpendicular, the data are compatible only with arrangement P. The extent of orientation indicated here, about 0.3, is substantially greater than the extent of orientation indicated in the flow dichroism studies discussed below and those of Cavalieri *et al.* (1956), for comparable DNA concentrations. However, both measurements agree when account is taken of the differences in geometry and the form of the functions averaged in the two types of measurement.

TABLE 2

FRACTIONAL CHANGE DUE TO FLOW IN THE FLUORESCENCE OF QUINACRINE BOUND TO DNA

Polarization of emission	X	X	Z	Z
Wavelength of excitation	3000	4500	3000	4500 Å
Exciting radiation Y polarized	+0.24	+0.35	−0.12	−0.11
Exciting radiation Z polarized	−0.10	−0.10	−0.26	−0.29

The DNA (as nucleotides) and total quinacrine concentrations were $3.76 \times 10^{-4}M$ and $5.5 \times 10^{-6}M$, respectively, corresponding to one quinacrine molecule per 34 nucleotide pairs. The shear rate during flow (at the walls of the capillary) was about 1.2×10^4 sec^{-1}. The X and Y polarizations are perpendicular to flow; Z is parallel to flow. The transitions excited at 4,500 Å and 3,000 Å are parallel and perpendicular, respectively, to the transition of emission.

Since the longest wavelength of excitation always corresponds to the same electronic transition as that responsible for emission, it will be possible in general to establish the angle between this transition and the helix axis for any fluorescent molecule that binds to DNA. Perpendicular transitions, however, are not always available at useful intensities, so that complete specification of the orientation of the plane containing the dye is not always possible. For substances having a positively polarized band with a substantially lower degree of polarization than the longest wave-length absorption, the calculation can also be made on the assumption that the lower degree of polarization results from transitions in more than one direction in the plane of the molecule. Acridine yellow, the most powerful mutagen studied by Orgel and Brenner (1961), provides a band at 305 mμ with degree of polarization, 0.10, when bound to DNA, as well as the long wave band in the region of 460 mμ with degree of polarization, 0.35. In flow experiments with acridine yellow like those described above, the fluorescence intensities resulting from excitation of either band again change by the same amount, and in the same directions as found for quinacrine. Since the perpendicular contribution to short wave-length excitation is of course much less, the precision with which the plane can be specified is correspondingly diminished, but the conclusion remains the same; the dye must be nearly perpendicular to the helix axis.

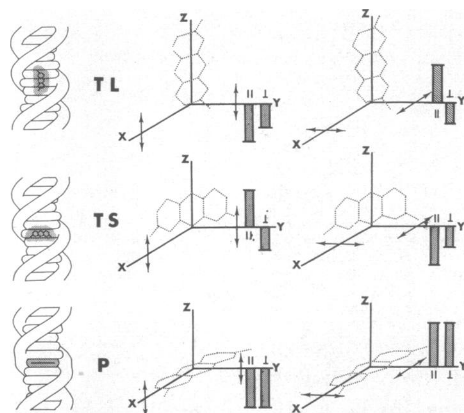

FIG. 2.—Three conjectures for the orientation of acridine bound to DNA. Each arrangement is identified similarly in Table 1. The bar graphs on each set of coordinate axes represent qualitatively the expected change in intensity of fluorescence when the DNA is itself oriented by flow along the Z axis. It is assumed that a beam of monochromatic light along the X axis, polarized as shown in each diagram, is absorbed by a transition in either the long or short axis of the molecule, according to its wavelength, and that the emitting transition is along the short axis. The light emitted in the Y direction is measured through a polarizing prism, directed as shown. The change corresponding to short axis excitation is identified as $\|$, and to long axis excitation, as $\perp$. (The acridine skeleton in the figure is that of proflavin.)

Orientation of the Plane of the Base Pairs.—The similarity of the configuration of pure, undenatured DNA in solution to the B structure found in fibers at relatively high humidity is usually accepted on the basis of two kinds of evidence: the continuous swelling of fibers at very high humidities, approaching the dissolved state, without any indication of an abrupt structural transition, and the determinations of mass per unit length and helix radius by low-angle X-ray scattering from liquid crystals and concentrated solutions of DNA (Luzzati *et al.*, 1961a). On this assumption the ambiguity of flow dichroism measurements on simple DNA solutions is resolved, and the determinations can be taken to signify only the extent to which the helix is oriented by flow, rather than the inseparable combination of helix orientation and base pair tilt. The question of tilting of the base pairs is again of interest with respect to the DNA-acridine complex, since strong tilting would lower the mass per unit length enough to allow for a net decrease even after the mass of the bound acridines is added (Lerman, 1961). Since the amount of tilting required,

roughly 45°, abolishes the dichroism of the helix in stretched DNA fibers (Wilkins *et al.*, 1951), the measurement of the flow dichroism of the DNA-acridine complex can be expected to respond sensitively if this structural change is involved in formation of the complex. If, on the other hand, the intercalation hypothesis is more nearly correct, the DNA dichroism should be about the same in the complex as in the pure state, or even somewhat greater because of the straightening of the helix indicated by the viscosity enhancement at low rates of shear (Lerman, 1961). Flow dichroism measurements on DNA and complexes with quinacrine are presented in Table 3. Similar results have also been obtained with proflavine. Di-

TABLE 3

FLOW DICHROISM OF DNA AND DNA-QUINACRINE

Wavelength	2600	4325	4550 Å
DNA only			
Dichroism, Z polarization	−0.19		
Dichroism, Y polarization	+0.086		
DNA-quinacrine			
Dichroism, Z polarization	−0.24	−0.25	−0.24
Dichroism, Y polarization	+0.11	+0.12	+0.11
Absorbance, DNA contribution	0.654	0.000	0.000
Absorbance, DNA plus bound quinacrine	0.989	0.106	0.100
Absorbance, total	1.422	0.240	0.213

The dichroism is tabulated as the fractional change in absorbance of the DNA or the DNA-quinacrine complex when the solution is subjected to flow. The shear rate at the windows was 1.18×10^4 sec^{-1}. The absorbances in the stationary solution show the distribution of absorption between DNA, bound quinacrine, and the unattached dye. The DNA concentration (as nucleotides) when measured alone was 3.2×10^{-3} *M*, and in the complex, 3.6×10^{-3} *M*. The quinacrine bound to DNA was 0.661×10^{-3} *M*, or one quinacrine molecule per 2.7 nucleotide pairs. The Y and Z axes are perpendicular and parallel, respectively, to the direction of flow.

chroism is given as the fractional change in absorbance due to flow, with the polarizing prism either parallel or perpendicular to the flow axis. It should be noted that the geometry is different than that of the flow fluorescence experiments; the flow is between parallel planes (the windows of the cuvette) rather than through a tube of circular cross section. Light will be absorbed in these solutions both by the complex and by unattached quinacrine, which will not contribute to the flow dichroism. Since it was desirable to approach saturation of the binding sites, the concentration of free quinacrine is considerable, and accordingly, it is necessary to deduct its contribution to the absorbance of the solution for correct estimation of the dichroism of the complex. For this purpose, the concentration of bound quinacrine was calculated from the ultraviolet absorption spectra, which differ significantly for the free and bound dye. The dichroism at long wavelengths, due entirely to the dye, will directly indicate the contribution of the dye to the dichroism at 2600 Å, since both transitions are in the plane of the rings. Since the dye accounts for only 0.34 of the absorbance of the complex at 2600 Å, the dichroism due to DNA in the complex must be very nearly the same as that of the dye.

Thus, all strongly tilted models are excluded since DNA is more, rather than less, dichroic in the complex than alone. Also, the close similarity of the dichroism values for the quinacrine and the DNA itself demonstrates that the plane of the purine and pyrimidine pairs must be parallel to the direction of the long wave-length transition of the quinacrine. In view of the preceding considerations, the planes of both must be parallel.

Structure of the Complex.—The fluorescence and dichroism results require that

both the base pairs and the acridine lie substantially perpendicular to the molecular axis, but do not necessarily specify the intercalated configuration. The additional restriction that the mass per unit length of the complex is less than that of DNA alone (Luzzati *et al.*, 1961*b*, Lerman, 1961) is in agreement with the properties of the intercalated model; it could be reconciled with arrangements based on external edgewise attachment of the acridine by the *ad hoc* supposition that gaps, filled with solvent, are generated in the stack of base pairs. Roughly one such gap, the thickness of a base pair, would be required for each acridine bound, so that at the saturation region for strong binding of proflavine there would be a gap for every two base pairs. This model is, of course, energetically highly implausible, and the supposition of an almost totally aqueous environment for the acridine is incompatible with the properties of the complex as determined by reaction rate studies (Lerman, 1963). The comparison of the rates of diazotization of the amino groups of proflavine and other acridines when bound to DNA and free in solution shows a severe restriction on the accessibility of the amino groups to attack by the nitrosating reagents. This restriction is not found when the amino acridines are bound to other anionic polymers, even where there is a broad aromatic moiety for face to face contact with acridine. The intercalated structure remains as the only hypothesis fully compatible with the diverse experimental evidence.

A Conjectural Mechanism for Mutagenesis.—A mechanism based on the intercalation of an acridine into a single strand of the DNA molecule, as proposed by Brenner *et al.* (1961) for generating insertion and deletion mutations during DNA synthesis, demands a somewhat different structural basis than the model proposed here, in order to accommodate the pairing of an extended strand containing the acridine with a nonextended strand containing one additional base. It has seemed more useful to consider whether a mechanism might be devised on the basis of the simple intercalated model, and taking into account also some of the other characteristic properties of acridine mutagenesis. Aside from the presumably irrelevant induction of mutations by photodynamic action of the acridines, it appears clear that there is a potent mutagenic effect in the reproduction of the T-even phages, and that there is no significant mutagenic effect on *Escherichia coli* during vegetative growth (as shown by our own observations and other investigators). There are at least two differences between the multiplication of bacteria and the multiplication of phage; the phage DNA contains hydroxymethyl cytosine instead of cytosine, and phage multiplication is accompanied by extensive genetic recombination. It is difficult to attribute any particular significance to the presence of the substituted cytosine that might be relevant to the acridine effect. (We have observed the usual viscosity enhancement in T2 DNA mixed with proflavine.) However, it is easy to attribute a role in mutagenesis to the frequent recombinatorial events that accompany phage multiplication.

Let us suppose that recombination takes place within a short region of the chromosome corresponding to the effective pairing, or switch, region, and that a few acridine molecules are intercalated at random in one or both of the paired chromosomes. Since the length along the chromosome occupied by an acridine is the same as the length required by a base pair (3.4 Å), wherever there is an intercalation in one of the chromosomes without intercalation at exactly the same place in the other, the pairing will be shifted exactly one step out of register at one side

of the site of intercalation. If the chromosomes are paired in perfect homology below the site of intercalation, the chromosome containing the acridine will offer the base pair corresponding to the next earliest neighbor to the other for pairing above the site of intercalation. After any interval in which the same number of acridines are intercalated into both chromosomes they will return to perfect homology. Within the interval they can be one, two, or more steps out of register in either direction. If the chromosomes break at the same position, side by side, and reunite with the exchange of partners, it will be seen that the reunion will result in the omission of one or more base pairs in one product and the corresponding duplication of one or more base pairs in the other product of the reunion. This mechanism thus has the property of generating insertion and deletion mutations, as required in the analysis of the interactions of suppressor mutations by Crick and collaborators (1961), and will be dependent on the frequency of crossing-over. Mutants will be expected to be recombinant for closely linked outside markers. The same process, crossing-over between paired chromosomes that are displaced from perfect homology by one, or a few, nucleotide pairs, could also be expected to occur spontaneously with the same results. It has been suggested (Fresco, personal comm., 1960) that the spontaneous process proceeds through the formation of short loops of one or more nucleotides excluded from the double helix, as suggested by the stoichiometry pairing of synthetic polynucleotides (Fresco and Alberts, 1960). The occurrence of recombination errors during meiotic division could account for the increased rate of spontaneous mutation found by Magni and Von Borstel (1962) in ascospores as compared with the mutation rate during vegetative growth of yeast. A formal mechanism for spontaneous mutation by unequal crossing-over has also been advanced independently by Demerec (1962).

Summary.—New physical evidence is presented bearing on the structure of the DNA-acridine complex. The results are fully compatible with the intercalated model, and incompatible with other plausible models. A mechanism based on recombination errors is proposed for the production of insertion and deletion mutations by intercalation of acridines.

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*CHANGES IN THE DISTRIBUTION OF POLYMERASE ACTIVITY DURING DNA SYNTHESIS IN MOUSE FIBROBLASTS**

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The availability of partially synchronized cultures of mammalian cell lines[1, 2] provides a new opportunity to investigate the control of DNA synthesis in the cells of higher organisms, which should supplement the extensive studies with lower forms.[3] In this paper will be described the extent and timing of DNA synthesis in mouse fibroblasts (L cells) partially synchronized with 5-fluorodeoxyuridine[4] as well as changes in the intracellular distribution of DNA polymerase activity which suggest that the particulate polymerase may be of more importance than recognized previously. During the course of this work, we learned that results similar to ours have been obtained by Gold and Helleiner.[5] Recently, Billen has described a polymerase-DNA complex in *E. coli*.[6]

Experimental.—L cells were grown in suspension as described previously.[4] Cell counts were obtained with the Coulter electronic counter model A; after preliminary studies an aperture current setting of 3 and threshold of 20 were used routinely. Such cell counts averaged 6 per cent greater than simultaneous chamber counts.[7]

The rate of incorporation of hypoxanthine-8-C^{14} (California Corporation for Biochemical Research) into DNA was determined by incubation of duplicate 1 ml aliquots of the suspension culture with a saturating concentration of hypoxanthine-8-C^{14} (3.7×10^{-5} *M*; 7.6 μc per μM) for 1 hr at 37°C in a Dubnoff shaker. Then the cells were diluted with 4 ml of cold 0.14 *M* NaCl solution and collected by centrifugation; the acid-soluble material was extracted with cold 5 per cent trichloroacetic acid and the residue digested with *N* NaOH for 16 hr at 37°C to hydrolyze RNA. After cooling, DNA and protein were precipitated with acid, collected by filtration on a cellulose filter (Millipore Filter Corporation; type AA filter with pore size 0.8 μ), washed four times by filtration of 5 ml of cold 5 per cent trichloroacetic acid, and assayed for radioactivity as described previously for thymidine-2-C^{14}.[4] All samples were counted to less than 5 per cent error. Essentially all incorporated radioactivity became acid-soluble if the cells were treated with 5 per cent trichloroacetic acid at 90°C for 20 min.

For the usual assay of DNA polymerase activity, 3×10^6 cells were collected by centrifugation, suspended by agitation in 1 ml of cold buffer containing 0.1 *M* KCl and 0.02 *M* tris(hydroxymethyl)aminomethane (pH 7.4), and transferred to a small cellulose test tube. After centrifugation at 4°C, the cells were suspended in 100 μl of cold 0.02 *M* tris(hydroxymethyl)aminomethane (pH 7.4), disrupted by vibration at 10 kc per sec for 2 min in a Raytheon Model DF 101 sonic oscillator, and separated into a supernatant fraction and a pellet by centrifugation at about $6{,}000 \times g$ for 30 min at 4°C. In this paper, the pellet will be referred to as the particulate fraction, although it is recognized that its components have been altered by sonic oscillation. The particulate fraction was washed once with cold 0.02 *M* tris(hydroxymethyl)aminomethane and suspended in 50 μl of the same buffer. The concentration of protein in each fraction was deter-