DNA was observed when mitochondria were isolated from brain and heart of sheep, and liver and heart of chicken; furthermore, mitochondria from guinea pig liver, which banded at two discrete densities in a Ficoll gradient, each showed DNA of density 1.701 g/cm^3.

Some experiments were also carried out to calculate the amount of DNA present in mitochondria of mammalian tissues in relation to mitochondrial proteins; the diphenylamine reaction (*22*) was used to evaluate the amount of DNA, and the method of Lowry *et al.* (*23*) was used to estimate mitochondrial proteins. The results obtained in the mitochondrial fractions of mammalian tissues were in the range of 4 to 5 μg of DNA per milligram of mitochondrial proteins. The amount of DNA present in a beef-heart mitochondrion was estimated as approximately 5×10^{-11} μg based on its reported protein content of 1.1×10^{-13} g by Green and Oda (*24*). This should correspond to a molecule of DNA with a molecular weight of 3×10^7 if all the DNA in a mitochondrion is present as a single molecule.

In the light of previously reported results and those reported here, we can conclude that the unique density in CsCl gradients (of mitochondrial DNA in simpler organisms) and the ability to renature in solution are two useful criteria to identify mitochondrial DNA. The density of mitochondrial DNA is different from the density of nuclear DNA in simpler unicellular organisms, molds, yeasts, algae, and protozoa, while it is apparently the same or higher in mammalian species. It is not known whether the unique density of mitochondrial DNA corresponds to the presence of a unique base, nor is it known whether the extent of methylation of adenine and cytosine is the same as in nuclear DNA. It was shown by Ray and Hanawalt (*25*) that the methylcytosine content of DNA from the chloroplasts of *Euglena gracilis* was lower than that of nuclear DNA. The most likely explanation for the ability of mitochondrial DNA to renature is its much greater homogeneity, compared with the nuclear DNA.

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Organization of DNA in Dipteran Polytene Chromosomes as Indicated by Polarized Fluorescence Microscopy

Abstract. *Analysis of the polarization of fluorescence from* Drosophila virilis *polytene chromosomes stained with acridine orange suggests that the DNA in the interband regions of these chromosomes cannot be in a supercoiled configuration, but must lie parallel to the chromosome axis.*

Recent studies have shown that the mode of binding of aminoacridine dyes to DNA in vitro, when the ratio of dye to nucleotide is about one to four or less, is by intercalation: that is, the planar dye molecule is sandwiched between the two adjacent base pairs inside the DNA helix (*1–3*). For higher ratios, a new mode of binding appears; the dye molecules are "stacked" along the outside of the helix with their planes perpendicular to the helix axis (*3, 4*). In both of these binding modes, the planes of the fused rings of the dye molecules are parallel to the planes of the purine-pyrimidine rings. The acridines are well-known fluorescent dyes, and in such planar molecules appreciable absorption will occur only if the direction of the electric field associated with the impinging light (that is, the *E*-vector) is not perpendicular to the plane of the rings. Similarly, the emitted fluorescence will have maximum intensity where the *E*-vector is parallel to the plane of the dye molecule. Therefore, if an acridine dye is bound to DNA and the complex is oriented, there will be one direction of polarization for which relatively little light will be absorbed or emitted. Lerman utilized this fact to support the intercalation model by orienting acridine-stained DNA in a flow gradient (*2*).

Using the dye acridine orange, which has a high specificity for nucleic acids in cellular preparations (*5*), we are taking advantage of this polarization phenomenon to analyze the orientation of DNA in biological material. This report deals with our preliminary results with the salivary gland chromosomes of *Drosophila virilis*.

The instrument is built around a fluorescence microscope equipped with a Zeiss oil-immersion objective of numerical aperture 1.32 (magnifying power, × 100) and a Spencer dry lens of numerical aperture 0.5 (magnifying power, ×20) inverted as a condenser. In front of the Hg-arc light source are two glass plates, tilted to act as reflection polarizers that correct for the slight inherent polarization of the light source. The plates are adjusted so that the light striking the specimen is completely unpolarized for those wave-

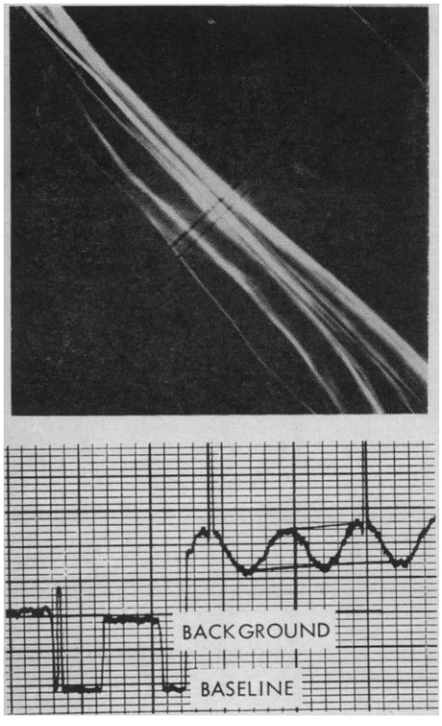

Fig. 1. Photograph and corresponding trace for *Drosophila virilis* salivary gland chromosomes which have been treated with trypsin prior to squashing. The crosshairs define the area being analyzed, and the reference spike occurs when the polaroid is parallel to the pair of crosshairs which goes from lower left to upper right.

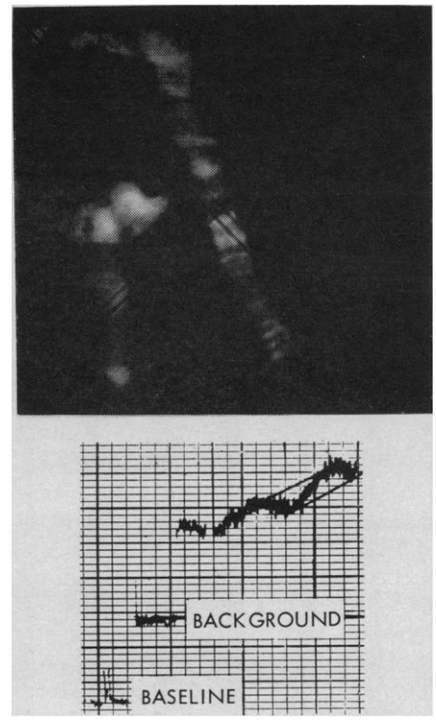

Fig. 2. Photograph and corresponding trace for *Drosophila virilis* salivary gland chromosomes which have been squashed in acetic acid without trypsin treatment. A large rate of signal decay during illumination is present, probably due to an availability of oxygen for photooxidation of the dye by the exciting light.

lengths at which the bound dye absorbs. The ocular is a Leitz × 10 with adjustable defining crosshairs built in. Light leaving the ocular is focused onto an image plane, passing first through a single polaroid filter which is motor driven (2 rev/min). At the image plane is an iris diaphragm, collimated with the defining crosshairs, which can be closed down to give a reading for a very small region of the object. Beyond the iris diaphragm, the light intensity is read by a photomultiplier tube (RCA 1P21) and amplified and recorded on a chart recorder. Swingout mirrors above the ocular allow the object to be seen and photographed through side tubes. Once in every revolution of the polaroid filter a reference spike is recorded on the chart recorder. Therefore, for aligned material, an oscillatory trace is recorded, and the percentage of polarized light (R) is calculated as the difference between maximum and minimum intensity divided by maximum intensity times 100. These intensities are corrected for background from fluorescence from the secondary filter and the diffuse fluorescing dye throughout the mounting medium. This background is measured on each side of the specimen region under analysis, averaged, and subtracted. There is a gradual decay in the intensity of the emitted signal due to bleaching of the bound dye by the exciting light.

It can be shown, by projecting the planar areas of the variously oriented dye molecules against the viewing plane and integrating the contributions from such projections, that for an nth order aminoacridine stained DNA supercoil lying across the field of view, the maximum value of R (in percent) obtainable is

$$R_n = (-1)^n(1/2)^n(100),$$

in which the DNA helix itself is considered the zero-order supercoil, and the $(-1)^n$ factor indicates that, for even n, the direction of polarization for minimum light intensity is parallel to the supercoil axis. Thus for simply parallel DNA, the maximum theoretical R_0 is +100 percent; for a first-order supercoil, maximum R_1 is −50 percent; for a second-order supercoil, maximum R_2 is +25 percent, and so forth. These values are only obtainable for "ideal" experimental conditions, in which there is uniform illumination of the dye molecules regardless of their orientation (that is, a condenser of extremely high numerical aperture), and only fluorescence which is emitted parallel to the optical axis is analyzed (that is, the numerical aperture of the objective is very low). For real experimental conditions, these factors can somewhat reduce R (by about 20 percent for a zero-order coil). In addition, such factors as depolarization at lens surfaces, and lack of perfect ordering of both dye and DNA helix will further reduce R from the theoretical maximum. The sign of R will not be affected by such factors, and it is this sign which plays an important role in our choosing among the different orders of coiling.

By treating the salivary gland in trypsin (0.4 percent in physiological saline) for 30 minutes and then squashing it, then treating with ribonuclease (0.5 mg/ml) and staining at a dye concentration of $10^{-3}M$, typical polytene chromosomes are replaced by bundles of uniform filaments, whose width is less than the resolving power of the microscope (2000 Å or less) and which are often stretched out to lengths twice the lengths of the normal chromosomes (Fig. 1). The number and configuration of the bundles make it seem likely that the filaments are the individual strands of the polytene chromosomes, separated laterally and stretched out by removal of the "slack" of the band and puff regions, each bundle corresponding to a single chromosome. Further trypsin treatment does not result in degradation of the basic filaments; deoxyribonuclease treatment removes them entirely. Analysis of the fluorescence polarization of groups of filaments from 12 randomly selected chromosomal bundles gives an average of $R = +46.4 \pm 2.3$ percent. The direction of minimum light intensity is parallel to the filament axes, so that n must be even, and the degree of polarization indicates that n must be zero, that is, the data fit a parallel array of uncoiled DNA helices.

Analysis of polytene chromosomes normally squashed in acetic acid, treated with ribonuclease, and stained in $10^{-3}M$ acridine orange (Fig. 2) gives the following results: bands and puffs, R less than 5 percent; interbands, $R = +20.2 \pm 5.0$ percent for 12 random interband regions, and several of the

interband readings were above 30 percent. As with the filaments, the direction of minimum intensity is parallel to the chromosome axis, within ± 5 degrees. Considering the size of the effect and the direction, the results are consistent with the interband regions of polytene chromosome, being a parallel array of uncoiled DNA. Presumably, in the band and puff regions, the DNA filaments are so tangled and looped as to preclude observation of any dominant orientation (*6*).

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Synchronization of Mammalian Cells with Tritiated Thymidine

Abstract. *Short exposures of mammalian cells to tritiated thymidine of high specific activity destroys the proliferative capacity of mammalian cells. Since the killing is limited to cells that have synthesized DNA in the presence of the labeled compound, an exposure duration of less than one generation can yield a synchronized population.*

The potential usefulness of synchronized or partially synchronized mammalian cells for the investigation of various cellular properties has long been recognized, and several different techniques have been used to produce such populations (*1–7*). In several of these the cell was subjected to nonphysiological conditions for a certain period, and this may very well have resulted in alteration of the processes being studied in the synchronized population. For example, synchronization achieved by fluorodeoxyuridine (FUdR) treatment and subsequent reversal with thymidine (TdR) leads to conditions of unbalanced growth (*2, 3*). The method originated by Terasima and Tolmach (*4*) and later modified by Sinclair and Morton (*5*) and Robbins and Marcus (*6*) does not appear to subject the cells to nonphysiological conditions, but the yield of cells which can be easily obtained is limited. In this report we outline an alternative method of producing large populations of partially synchronized populations of L-cells based on the use of tritiated thymidine (H^3TdR).

The incorporation of H^3TdR into the DNA of mammalian cells can destroy their capacity for continuous proliferation (*8*). Therefore it seemed possible to produce a synchronized population of mammalian cells through the use of H^3TdR of high specific activity. The basis of the method can be understood with reference to Fig. 1. Immediately upon addition of the label to an L-cell population in which the durations of G_1, the phase prior to DNA synthesis was 5 hours; of S, the DNA synthetic phase, 8 hours; of G_2, the phase following DNA synthesis, 4 hours; and the mitotic phase M, 1 hour, all cells which were actively synthesizing DNA (that is, S-phase cells) should immediately take up the label. If the label were left in the culture for a period of 3 hours, then cells would flow from G_1 into S and become labeled, and labeled cells should flow from S into G_2. If the exposure to the H^3TdR were extended to 6 hours, then all of S, G_2, M, and part of G_1 would be filled with labeled cells, and the only unlabeled cells would be those few in a narrow phase interval at the end of G_1. If the concentration of H^3TdR and the specific activity were sufficient, then all of the labeled cells should have lost proliferative capacity, and the only viable cells in the population would be those few cells at the end of G_1. If at this time a large quantity of unlabeled TdR were added, then the cells at the ends G_1 would not take up label, and they would remain viable, constituting a partially synchronized population.

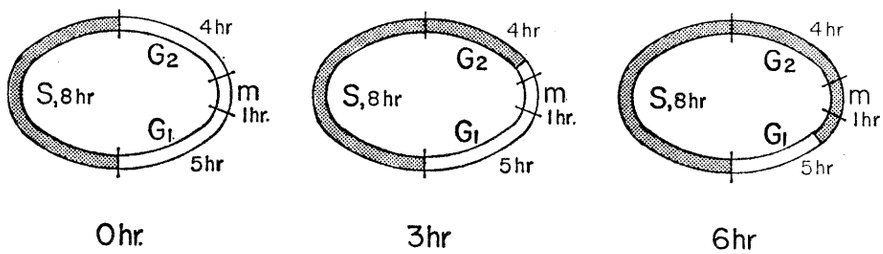

Fig. 1. Scheme of the cycle of L60T cells, showing the fraction of the cycle occupied by labeled cells after a short exposure, a 3-hour exposure, and a 6-hour exposure to H^3TdR.

For the method to be successful it must be possible to incorporate sufficient radioactivity into the cell in a short time to bring about the loss of proliferative capacity, and it must be possible to terminate immediately the uptake of labeled thymidine by the addition of unlabeled thymidine. Also the amounts of H^3TdR or of TdR must not affect cells in the population which have not incorporated the labeled material. Our experiments indicate that for mammalian cells in vitro all of these conditions can be satisfied.

We used L60T cells, a subline of L cells adapted to growth in a medium containing no exogenous thymidine, but capable of using an exogenous source when supplied (*3*). They were grown in suspension culture in spinner flasks at 37°C in growth medium 1066T—, consisting of 1066 (Connaught Medical Research Laboratories)