loss of polarization can be caused by 'extrinsic' factors such as migration of excitation (ET) and brownian movement[10]. Thus, the λ term of the equation is the 'intrinsic factor' while the term containing ω is the 'extrinsic factor'. The calculated values of λ for SBF are uniformly 12–15° higher than that of F in the excitation range of 270 nm to 305 nm where F emission is monitored at 306 nm and SBF at 324 nm. Also the average value of ω in F going from excitation at 306 nm to excitation at 285 nm is calculated to be 25°. On the other hand, the SBF data (excitation at 285 nm) show a higher ω value (about 35°) in the 320–350 nm range and very high values (37°–78°) in the 370–390 nm range. We interpret these data to mean that the higher wavelength region of the SBF fluorescence band excited at 285 nm is more susceptible to polarization changes than the lower energy part of the band.

The extrinsic contribution to depolarization increases by a factor of 2 in going from 330 to 340 nm by a factor of 3 in going from 330 to 380 nm. The additional depolarization from extrinsic causes must occur through ET between the neighboring intramolecular chromophores. Thus to Weber's 2 categories of extrinsic contributions to depolarization (intermolecular ET via dipole interactions and brownian movement) can be added a 3rd category: intramolecular ET via dipole interactions, which plays an important role in the increasing of depolarization in SBF.

Differential banding induced in polytene chromosomes of *Drosophila melanogaster* stained with acridine orange

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Summary. Experiments carried out on polytene chromosomes of *Drosophila melanogaster* showed differential staining of certain areas when cytological preparations were exposed to light, or treated with formaldehyde, and subsequently stained with acridine orange. Some hypotheses are discussed regarding the involvement of some proteic fractions which, in addition to DNA, could pay a part in such banding.

It is known that eukaryotic chromosomes exhibit patterns of transverse fluorescent banding when stained with some acridine derivates[2]; the initial interest in such banding, as simplifying the recognition of particular chromosomes, was followed by that regarding the linear structure of eukaryotic chromosomes due to possible interaction of chromosomal DNA with fluorochromes. It is in fact known that some of these compounds, when bound to specific polynucleotide DNA sequences, enhance their fluorescence, characterizing specific base compositions. Quinacrine, for example, was shown to be an indicator of base compositions in polynucleotide segments: AT-rich sequences enhance while GC-rich sequences quench Q- fluorescence[3, 4], even if the specific interspersion of GC base pairs in AT-rich segments seems to play a role in quenching of in vitro fluorescence of this compound[5]. Hoechst 33258, an alkaline bi-benzimidazol derivative, is another indicator of AT-rich polynucleotide sequences; the fluorescence in vitro of this compound is enhanced by both AT- and GC-rich segments, though the AT-rich sequences increase the fluorescence more than GC[6, 7]. It ist still uncertain, however, if it is possible to make the same assumptions about the behavior of such fluorochromes, either with DNA in solution or when it is part of complex structures like eukaryotic chromosomes[8, 9].

In contrast to the above-named compounds, acridine orange (a.o.) fluorescence is not increased by specific base sequences, since for this compound both $(dA)_n$ $(dT)_n$ and $(dG)_n$ $(dC)_n$ enhance fluorescence in vitro[10]. Chromosomes stained with this fluorochrome show bright yellow-green fluorescence all along their length. Because the a.o.-DNA bond seems to be intercalating, the emission of yellow-green fluorescence could be considered to be the result of the a.o.-DNA complex when all the spaces between pairs of adjacent bases of the nucleid acid in its double helix state are saturated with fluorochrome molecules[11, 12]. There is, however, emission of fluorescent light with a wavelength characteristic of red-orange when the dye molecules interact with each other. In this connection, it is important to emphasize that treatments producing depolymerizations, in addition to denaturation of DNA, can also cause the fluorescence to change from yellow-green to red when the nucleic acid is bound to a.o.[13].

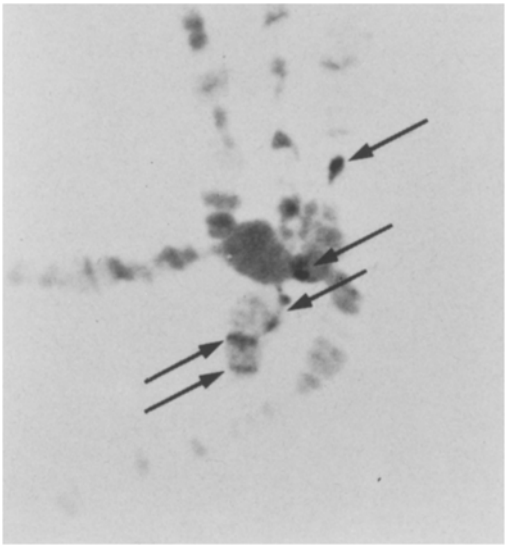
In thus seems possible to differentiate, with reasonable certainty, double helix polynucleotide sequences producing yellow-green fluorescence, from single helix sequences emitting red fluorescence. Because of the similar behavior of the DNA in fixed chromosomes and in solution[11], one can consider the a.o. as an indicator for characterizing form and dimensions of the nucleic acid in chromosomes. In this paper we describe the a.o. differential staining obtained after light and/or formaldehyde treatment on polytene chromosomes of *Drosophila melanogaster*. Some hypotheses on the meaning of the fluorescent banding are proposed.

Materials and methods. 3rd instar larvae of *Drosophila melanogaster* (Canton S) raised at the Zoology Department of the University of Wisconsin were used. The salivary glands were extracted in Ringer solution and were then treated with 45% acetic acid for 3 min. After squashing, the siliconized coverslips were removed with a razor blade from slides immersed in liquid nitrogen; fixation was effected in 95% ethyl alcohol for 10 min. Slides were stained with 0.01% acridine orange in Sorensen phosphate buffer M/15, pH 7.1 for 15 min. The excess dye was removed by a 30 min treatment in the same buffer solution. Preparations were then mounted in a drop of phosphate buffer, sealed with fingernail polish and exposed to the light of a 14 W sterilizing mercury lamp (General Electric) for times varying from a few min to 10 days. Control slides were contemporaneously kept in the dark for the same times as those exposed to light. Some preparations were treated with 4% formaldehyde for 10 min before staining. Slides were observed daily.

Results and discussion. No action of the buffer solution was observed in the control slides; as already reported[14], a bright yellow-green color was present in all the polytene chromosomes of *Drosophila melanogaster* stained with a.o. The cytological preparations, mounted in buffer and exposed to the light for a period of at least 3–5 days, show the chromosome bands and interbands of a color varying from dull orange to brick red; however, some bands and some dots in the chromocenter are differentially stained and show a bright yellow-green fluorescence (figure). Such structures seem to be the same ones already shown with Quinacrine and 33258 Hoechst[8–15] and namely: band 8IF (3R), 3 bands on the 4th chromosome and some dots in the chromocenter area.

Our data, therefore, show that the chromosomes, after a.o. staining and exposure to light, exhibit a differential banding pattern on some bands which retain the yellow-green color in contrast to all other chromosomal areas where one notes a change from yellow-green to an orange/brick red color. Freifelder et al.[16] showed that purified DNA irradiated with visible light in the presence of a.o. results in depolymerization of the nucleic acid; on this basis our experiments could indicate either denaturation or depolymerization of DNA in most of the polytene chromosomal areas, with the exception of the few bright bands.

In order to find out if something other than DNA is involved in the mechanism of this banding, we treated some preparations with formaldehyde after acid fixation: the same a.o. differentiation shown after light was present immediately, even if the fluorescence was not so bright. The action of formaldehyde in stabilizing phage DNA is known[17]; this compound prevents reassociation of single polynucleotide chains after heat or high-pH denaturation. On the other hand, the stabilizing action of formaldehyde on deoxyribonucleoproteins is also known, as pointed out by Rigler et al.[18] on interphase limphocyte nuclei, and by Nash and Plaut[14] who showed that *Drosophila* polytene chromosomes treated with this compound, fixed in acetic acid and stained with a.o., always show a yellow-green fluorescence even after heat or acid treatment.



Differential staining of polytene chromosomes of *Drosophila melanogaster* stained with acridine orange and exposed to the light for 5 days. Band 8IF (3R), 3 bands on the 4th chromosome and some dots in the chromocenter are evident. (See arrows.) Such areas show a green color, while all the rest of the chromosome body is dull orange.

The possible involvement of proteins in differential a.o. staining of human chromosome preparations treated with formaldehyde was implied by Bobrow and Madan[19]. Bobrow[20], moreover, pointed out that human chromosomes treated with trypsin and stained with a.o. show a yellow-green/red banding pattern similar to R banding[21]. The data are interpreted by the author on the basis of differentiation due also to chromosomal molecules different from nucleic acids.

We are led to believe that, in our experiments, the action of formaldehyde takes place by stabilizing the DNA-protein and/or protein-protein structural relationships already altered by the acid fixative. The a.o. banding pattern obtained could be the result of interactions involving probably both DNA and some proteic class of the polytene chromosomes of *Drosophila melanogaster*. We do not know what the light energy absorbed by the a.o. can produce in the chromosomal entities to which the fluorochrome is bound, but we think that an appropriate use of the interactions light/a.o.-DNA could act as a powerful tool for further research on the structure of aukaryotic chromosomes.

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