of light. These fluoride-induced discrete waves resemble those normally produced by light. We tentatively conclude that fluoride induces a process similar or perhaps identical to visual excitation of the photoreceptor.

One naturally wishes to speculate as to the mechanism by which fluoride may act. We tested the effect of fluoride on the photoreceptor to investigate the possible role of cyclic nucleotides in photoreceptor physiology and to use a pharmacological agent that would increase cyclic nucleotide concentrations by activating cyclase, the enzyme that synthesizes cyclic nucleotides. Fluoride activates brain adenylate cyclase apparently by acting directly on the catalytic subunit of the enzyme (*11*). However, it should be kept in mind that we have no evidence to indicate that changes in cyclic nucleotide concentrations mediate the effects of fluoride. Fluoride is known to have other effects; for example, it is an inhibitor of a variety of metallo enzymes (*12*). One obvious candidate for the site of action of fluoride is the visual pigment of the photoreceptor. Fluoride might induce spontaneous isomerizations of the pigment chromophore and thereby lead to visual excitation. Whatever the mechanism of action of fluoride on the photoreceptor, it seems clear that fluoride will be a useful agent for further studies of visual transduction.

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7. The ASW contained 435 m*M* NaCl, 10 m*M* KCl, 10 m*M* $CaCl_2$, 25 m*M* $MgSO_4$, 20 m*M* $MgCl_2$, and 10 m*M* Hepes; *p*H 7.0.
8. The addition of 10 mmole of NaF per liter of ASW that bathed the preparation resulted in the effects described in this paper. In control experiments the addition of 10 mmole of NaCl per liter of ASW was without effect. Therefore we conclude that the effects seen were the result of fluoride addition.
9. Inasmuch as the effect of fluoride occurs in the dark, we can rule out fluorescence as a source for fluoride-induced discrete waves. It is possible (although unlikely) that the fluoride might induce bioluminescence, which would give rise to discrete waves. If such luminescence existed, it should be possible to measure the light produced. We were unable to measure any luminescence of the tissue when exposed to fluoride. Our measurement system was sensitive enough to detect a light stimulus that gave rise to discrete waves of similar frequency to those induced by fluoride.
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## Simultaneous Production of Q and R Bands After Staining with Chromomycin $A_3$ or Olivomycin

Abstract. *Human and mouse chromosomes, stained with either chromomycin $A_3$ or olivomycin, which bind preferentially to G · C-rich DNA (where G is guanosine and C is cytosine), exhibit a Q or a reverse banding pattern, depending on the wavelength used for excitation. The two complementary banding patterns can be observed in the same metaphase simply by changing the combination of excitation filters. These data suggest, therefore, that in addition to base composition, other factors are involved in the production of chromosome banding by chromomycin $A_3$ and olivomycin.*

Staining with fluorochromes that specifically bind to A · T-rich DNA (where A is adenine and T is thymine) produces a characteristic Q-banding pattern in human and other chromosomes (*1*). Some antibiotics, however, such as olivomycin, chromomycin $A_3$, and mitramycin, which bind preferentially to G · C-rich DNA (*2-4*), produce a reverse (R) banding pattern (*4-6*). Taken as a whole, these data support the hypothesis that variations in base composition along the chromosome are the major factors in Q and R banding.

We have investigated the cytochemical properties of chromomycin $A_3$ and olivomycin and have found that, depending on the wavelength used for excitation, these compounds can produce not only R banding but also a sharp Q-type banding pattern.

Standard chromosome preparations from short-term cultures of human leukocytes and from mouse fibroblasts (strain $DBA_2$) were stained for 20 minutes at room temperature with 5 $\mu$g (per milliliter) of either chromomycin $A_3$ (Boehringer) or olivomycin (Calbiochem) dissolved in phosphate buffer (0.15*M* NaCl, 0.03*M* KCl, and 0.01*M* $Na_2HPO_4$ adjusted to *p*H 7) containing $MgCl_2$ (2.5 m*M*). The slides were then washed and mounted in the same phosphate buffer at *p*H 7 and examined under a Zeiss fluorescence microscope equipped with incident illumination from a 200-W mercury light source. Two different combinations of dichroic mirrors and Zeiss filters were used for observation: (i) 2 × BG12 excitation filters, FT510 dichroic mirror and LP515 barrier filter (violet-blue excitation range); and (ii) UG5 plus BG3 excitation filters, FT460 dichroic mirror, and LP475 barrier filter (ultraviolet-violet excitation range). All observations were made with a Neofluar 100× Zeiss objective.

The observation of human metaphase chromosomes with combination (i) revealed that both chromomycin and olivomycin produce a specific green fluorescent banding pattern (Fig. 1a) that is very similar to the Q banding observed after staining with Hoechst 33258 (*7*). This was shown by the behavior of the secondary constrictions of chromosomes 1 and 16, which exhibited a bright fluorescence like that appearing after Hoechst staining. However, with combination (ii), we observed the yellow fluorescent R-banding pattern already described by van de Sande *et al.* (*4*). (Fig. 1b). The most surprising feature of this phenomenon was that the Q banding and the R banding could be observed and studied in the same metaphase simply by changing the combination of filters. Similar observations were made on mouse chromosomes stained with chromomycin $A_3$ or olivomycin (Fig. 2). With combination (i) the centromeric regions exhibited a bright fluorescence similar to that after Hoechst 33258 staining (*8*). With combination (ii), however, these regions were much less fluorescent than euchromatic arms.

We wish to point out that our staining technique, contrary to that described by van de Sande *et al.* (*4*), does not require a high concentration of the two antibiotics. The best results are obtained when the slides, stained with chromomycin $A_3$ or olivomycin (5 $\mu$g/ml), are kept for 24 hours in the dark at room temperature before observation. With this process of "maturation" a


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more intense fluorescence of both Q and R banding is produced and there is only a negligible fading.

The two complementary banding patterns are observed even if no $MgCl_2$ is added to the staining solution. In this case, however, fluorescent bands fade rapidly and it is difficult to take photographs.

Our results clearly show that both chromomycin $A_3$ and olivomycin can produce either a Q- or an R-banding pattern depending on the wavelength of excitation. Whereas the Q banding exhibits a green fluorescence, the R banding shows a yellow fluorescence. The simplest interpretation of these results is that the two antibiotics bind both the Q and the R regions, but with different chemical bonds. Chromomycin $A_3$ and olivomycin, bound to the Q regions, would be specifically excited with combination (i) producing a Q-banding pattern, whereas, bound to the R regions, they would be specifically excited with combination (ii) and would exhibit a reverse banding.

Although studies on DNA in solution indicate that chromomycin $A_3$ and olivomycin are specific G · C ligands (*2-4*), the present data show that, if they are excited with combination (i), these antibiotics specifically stain the A · T-rich centromeric regions of human chromosomes 1 and 16 [see (*9*)] as well as all the A · T-rich mouse centromeric regions (*10*). The benzimidazole derivative Hoechst 33258, which specifically binds A · T-rich DNA (*11*), produces a similar banding pattern with the same filter combination [combination (i)], but does not produce an R-banding pattern when excited with combination (ii) (*12*). Thus these three compounds produce a similar banding pattern on fixed chromosomes independently from their different affinity for A · T-rich or G · C-rich DNA in solution. These considerations suggest that the peculiar banding pattern produced by chromomycin $A_3$ and olivomycin is not simply related to variations in base composition along the chromosome.

In addition to the base composition, an important role in determining the banding specificity of these two antibiotics may be played by variations in base sequence (*13*) or in protein-DNA interaction along the chromosome (*14*). In this context it should be recalled (*14, 15*) that data from studies in vitro of DNA fluorochrome interaction do not permit a reliable prediction of the response of chromosomes to fluorochrome staining. Nevertheless, we think that further studies on the molecular interaction of chromomycin $A_3$ and olivomycin with the various chromosomal components may provide important insights into the chemical basis of chromosome banding.

Our results show that staining with chromomycin $A_3$ and olivomycin provides a banding technique with an extremely high power of resolution. The possibility of scoring two complementary banding patterns in the same metaphase makes this technique particularly useful in diagnostic studies with human chromosomes.

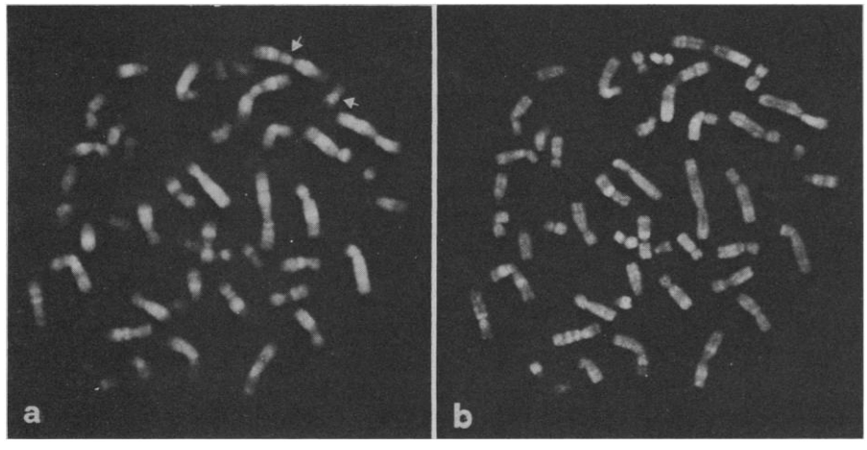


Fig. 1. Male human metaphase stained with chromomycin $A_3$ and observed (a) with combination (i) and (b) with combination (ii). The arrows indicate the secondary constrictions of chromosomes 1 and 16.

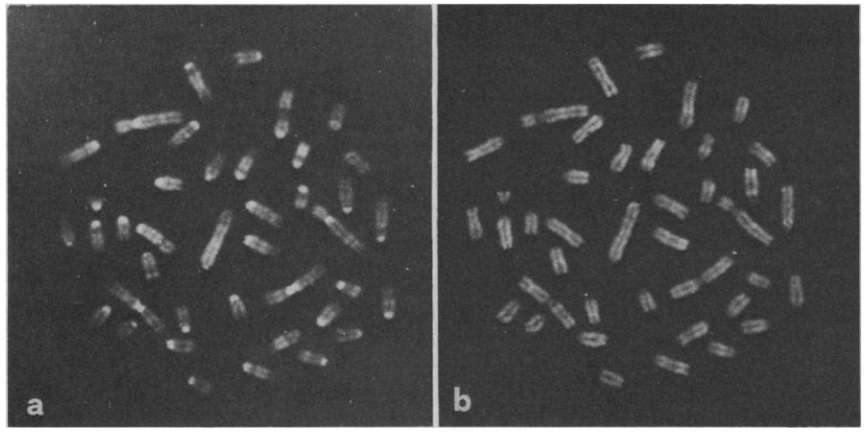


Fig. 2. Fibroblast mouse metaphase (strain $DBA_2$) stained with chromomycin $A_3$ and observed (a) with combination (i) and (b) with combination (ii).

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