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Fluorescent Staining of the Male Chromatin Body in Interphase Human Nuclei

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SUMMARY

A fluorescence staining method of the male chromatin body in human interphase nuclei is described.

The use of DNA-binding fluorescent agents in the study of mitotic chromosomes has been introduced by Caspersson et al (1968, 1969 a,b). When stained with quinacrine or its derivates, the Y chromosome is easily recognizable in human blood culture plates in metaphase by the keen fluorescence emanating from the distal end of its long arm (Zech, 1969; Vosa, 1970; Moscetti et al, 1971). With quinacrine dihydrochloride an intensely fluorescent body corresponding to the distal end of the long arm of the Y chromosome has been observed in cultured fibroblast and lymphocyte nuclei and in nuclei of the oral mucosa of normal males (Pearson et al, 1970).

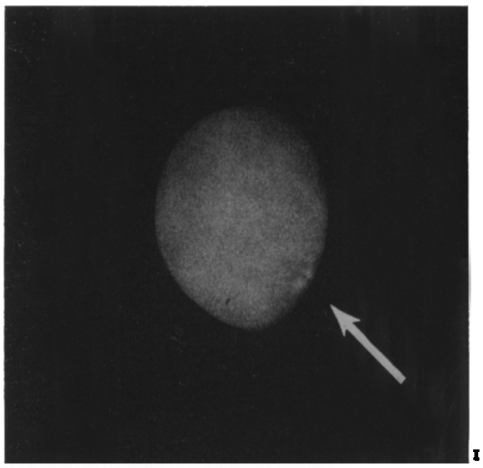
It has further been possible to evidence such body in human spermatozoa (Barlow and Vosa, 1970) and in cells deriving from the amniotic fluid of male fetuses (Caspersson et al, 1970).

We have made several observations on nuclei of the oral mucosa of normal males of various ages and on interphase nuclei of cultured lymphocytes, using 1-[(6-chloro-2-methoxy-9-acridinyl)amino]-3-(diethylamino)-2-propanol (Acranil Bayer), a substance extremely soluble in water as well as in methanol, and considerably irradiation-resistant.

The oral mucosa slides, as soon as taken, have been immediately fixed in methanol. Peripheral blood cultures have been prepared according to the procedure of Moorhead et al (1960).

The slides have been put for 8 min. in a 1% aqueous solution of Acranil (A), then washed in running wather from 3 to 5 min., and at last rinsed and mounted in a Walpole buffer at 5.6 pH.

The observations have been made with a Zeiss fluorescence photomicroscope. The light source was an " HBO200 " mercury vapor lamp; the excitor filters were Schott BG3/BG12 and the barrier filter was a " 53/44 ". The negative films were obtained with Ilford HP4 and prints with Agfa Gevaert Brovira 6.



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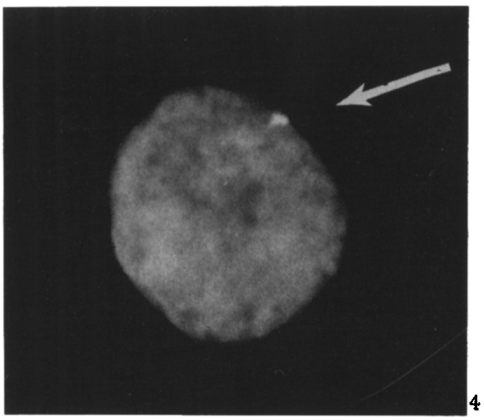

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Figures. 1-2: Oral mucosa nuclei showing the F-body.

3: An oral mucosa nucleus in which the F-body shows a double structure.

4-5: Nuclei of cultured lymphocytes. The arrows indicate the male chromatin body.

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With the A-staining we have been able to obtain, with a frequency ranging from 20% to 60% of the cells examined, very clear pictures of the F-body which in most cases is placed at the extreme periphery of the nucleus (Figs. 2-4-5), while sometimes it is observed more centrally with respect to the nuclear membrane (Fig. 1).

In a small percentage of cases the male chromatin body shows a double structure (Fig. 3). It is, however, easier to recognize the F-body in the nuclei of cultured lymphocytes rather than in the oral mucosa slides, because in the latter the presence of bacteria and of often overstained cytoplasm disturbs remarkably the observation. It is furthermore advisable that the slides be stained and observed through the fluorescence microscope as soon as prepared.

The search for fluorescent substance able to evidence more and more clearly the male chromatin body in human interphase nuclei is determined by the importance of the evidencing of such fluorescent body in the rapid determination of the genetic sex.

It will thus be possible to effect extensive surveys on large layers of the population, and particularly on groups of selected subjects, in order to determine possible bearers of numerical anomalies of the Y chromosome, with special reference to 47, XYY subjects. In fact, in the interphase cell-nuclei of such individuals it is possible to observe double fluorescent bodies (Pearson et al, 1970).

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Riassunto

Viene descritto un metodo di colorazione fluorescente del corpo cromatinico maschile nei nuclei umani in interfase.

Résumé

Description d'une méthode de coloration fluorescente du corps chromatinien masculin dans les noyaux humains en interphase.

Zusammenfassung

Beschreibung einer fluoreszierenden Färbmethode des männlichen Chromatinkörpers in der Interphase Zellkerne.

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