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Y-Fluorescence in Human Interphase Nuclei

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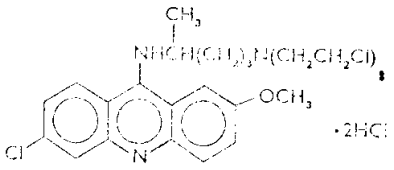
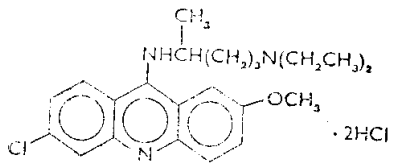
Introduction

In late 1968, a group from the Karolinska Institute, Stockholm, led by T. Caspersson, published the first of an astonishing series of papers. In the first paper (Caspersson et al. 1968) they described observations on the appearance of specific fluorescent banding pattern in chromosomes of Vicia and other plants using fluorescent acridine derivatives[1, 2, 3]. In November 1969, L. Zech, one of the Karolinska group, briefly mentioned that the distal portion of long arm of human Y chromosome is much more intensely fluorescent than any other chromosome region[4]. Subsequently in Oxford, Pearson and colleagues (1970) observed similar fluorescence using quinacrine dihydrochloride, which is more easily available[5, 6]. In the following year, Polani et al. (1971) attempted to apply the technique for the detection of the human Y chromosome in interphase nuclei[7]. According to them, application of this technique to clinical and chromosomal diagnosis and to the prenatal detection of the Y in amniotic cells seems at least as promising as has proved the earlier identification of the Barr body.

Our initial study was directed toward investigating the possibility of positively identifying male nuclei interphase by virtue of staining property of the Y. The purpose of this paper is to report our study, for the first time in this country, of evaluating the rate of appearance of Y fluorescence in interphase nuclei of normal male buccal smears and comparing the results with that in female subjects.



Quinacrine mustard dihydrochloride



Quinacrine dihydrochloride

Fig. 1. Fluorochromes

Methods and Materials

As for staining procedure, a precedent established methods were applied (Pearson et al. 1970; Polani et al. 1971; George et al. 1970)[5, 6, 7, 8]. The fluorescent dye mainly used was quinacrine mustard, but quinacrine

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dihydrochloride was also used (Fig. 1). Quinacrine mustard was used in stock solution because of difficulties in obtaining it, but other solutions were freshly made up before use. Quinacrine mustard in stock solution was found to remain active for at least a month when put in an ice box (4°C). Three staining solutions; quinacrine mustard buffer(Q.M.B.), quinacrine water (Q. W.), and quinacrine buffer (Q.B.) were used in our experiment.

A. Quinacrine mustard buffer:

Q.M. was made up at a concentration of 250-300 μg/ml in McIlvaine's buffer at pH 4.0-4.5. Slides were fixed in methylated spirit (95 per cent ethanol) and the fixed slides were allowed to dry, washed in buffer for 5 minutes, stained in Q.M.B. solution in 15 minutes, rinsed in buffer, and mounted in buffer. The coverslips were ringed with nail varnish.

B. Quinacrine dihydrochloride buffer:

Quinacrine dihydrochloride 0.5 per cent was used in deionized water. Slides were washed in deionized water, stained for 5 minutes, washed in running tap water for 3 minutes, mounted in buffer at pH 5.5, and also ringed with nail varnish.

C. Quinacrine dihydrochloride buffer;

Quinacrine dihydrochloride 0.5 per cent was used in McIlvane's buffer at pH 4.5. Slides were stained and thereafter rinsed in buffer and mounted in buffer. Nail varnishing was also used.

At first, the fluorescent technique was tried out with cells of known sex. Then 50 coded slides (male 25, female 25) were made up for this experiment. The experiment on coded slides was conducted and scored blindly with a count of 100 nuclei.

We used an AO (American Optical) fluorescence microscope. The light source was an "HBO 200" mercury vapour lamp with a 1.5 mm "BG 12" exciter filter and a yellow green (AO catalog no. 1110-602) or orange (AO catalog no. 1110-601) barrier filter. All observations were made using AO achromatic objectives (mainly×400 dry lens). Photographically we also used this system. Two kinds of black and white film of our choice were Kodak Panatomic-X (ASA 32) and Tri-X (ASA 400). Kodak Ektachrome 136 color film(ASA 64) was also used. Exposure time in slow film (Panatomic-X) is about 16 minutes, but in very fast film only 4 minutes.

Table 1. Y-fluorescence of interphase nuclei stained with each solution

Fluorochrome	Sex	No.	Y-fluorescence (%)				Comments
			+	±	−	Range of positivity	
Quinacrine Mustard	Male*	10	52	0	48	36-78	
Buffer	Female*	10	10	0	90	4-21	1 false (+)
Quinacrine	Male*	10	28	9	63	12-36	1 false (−)
Water	Female*	10	7	7	86	2- 9	
Quinacrine	Male*	5	34	6	60	10-44	1 false (−)
Buffer	Female*	5	8	7	85	6-16	1 false (+)

* Indicates that the difference is significant at the level of $p<0.005$ by x^2 test

Results

A small brightly fluorescent body could be easily observed in interphase nuclei of cells derived from male subject. The rate of appearance of Y fluorescence varied with each staining solution (Table 1, observer's score is pooled). The proportion of cells showing Y fluorescence with Q.M. varied from 36 per cent to 78 per cent in different preparations. The results with other staining solution is similar, but in general the rate is lower than that with Q.M. Scoring nuclei with Q.M., we had one false positive result. This slide was proved at the time of scoring to be of poor technical quality. The causes of other misclassifications (Q.W.: one false negative, Q.B.: one false negative and one false positive) proved to be due to the presence of many bacteria and poor technical quality. An average of 52 per cent from males showed Y-fluorescence with Q.M. as compared with 10 per cent among the females. The Q.W. treated buccal smears gave Y-positive results in 28 per cent of nuclei of males. Comparable figure in female buccal smears was 7 per cent. As for Q.B. treated slides, result from male was 34 per cent, but from female 8 per cent.

The Y-fluorescence may be seen anywhere in the nucleus. Compared with the case of the Barr body, the site is not characteristic. The size of the Y-fluorescence spot varies, but its brightness seems to remain the same.

Discussion

When using Q.M. and oral smears a count of 100 cells ought to be made to differentiate subjects who have Y containing cells from those whose cells have no Y. The technique of Y fluorescence staining in interphase is simple and reliable, but there are some technical difficulties. Firstly over-staining resulted in intense cytoplasmic fluorescence which obscured the nucleus. Loss of fluorescence due to overwashing is the second technical difficulty in identification of Y. Third, in interpreting oral smears which are heavily contaminated with bacteria, a lot of care has to be taken not to confuse the Y-body and bacteria. In addition, oral smears stored for 5 days or longer showed poor technical quality, V Photographically, we also had a great deal of trouble with exposure time.

The fluorescent technique is complementary to nuclear sexing of Barr and Bertrem[8]. It has a great usefulness to detect numerical abnormalities of the Y chromosome. It may also prove of value in the structural chromosomal aberrations involving the Y chromosome. The method is of interest in the study of interphase amniotic fluid cells[6, 7, 8, 9]. Our experience is limited to buccal smears, but further extensive trials seem to be promising. Antenatal sexing is already offered in the case of sex-linked diseases such as Duchenne muscular dystrophy or hemophilia, where selective abortion of males may be considered[9].

Kurnick and Radcliffe (1962) demonstrated that quinacrine combines with DNA in the stoichiometric proportions of approximately 1 dye molecule per 4 nucleotides[11]. In summary of literatures, quinacrine interacts with DNA by intercalation of the acridine nucleus in the double helix and by ionic binding of the diaminoalkane side chain to the phosphate groups. Quinacrine mustard

may interact similarly, and in addition by alkylation of DNA bases, in particular at the N-7 atom of guanine[1, 2, 3, 11, 12]. Pearson concluded that chromosomes were staining up differences in the base composition of DNA[6]. As subsequent events showed, this view has had to be modified. According to Commings (1971), binding of the quinacrine is determined by a protein moiety attached to the DNA helix[10]. But literatures on binding patterns of fluorochrome are scarce and they are still contradictory.

Pearson (1972) said that the physical dimensions of the male chromatin body described are similar to those of the fluorescent region of the Y chromosome[6]. That is to say, part of the Y chromosome is contracted in the interphase nucleus and this respect is analogus to the second X chromosome in female interphase nuclei. George (1970) pointed out that the characteristic pattern of the binding of Q. M. to the heteropychnotic distal portion of the Y chromosome and the abscence of any specific binding to one of the two X chromosomes in the normal female are intriguing[8]. Informations on the reason for the heterogeneous staining reaction between human chromosome and quinacrine are scarce, but it seems to reflect chemical difference in two chromosomes.

Caspersson et al. (1969) demonstrated that the quinacrine mustard fluorescence in the chromosome material is quite resistent to continued UV irradiation. Continued irradiation at the same intensity in the instrument led to no appreciable change in fluorescence during the first 3 minutes, followed by an increase of 10-20 per cent during the next 10 minutes and then a gradual decline[2]. In our experience, similar results were obtained. Illumination for more than 30 minutes made fluorescence fade out.

Summary and Conclusion

To evaluate the rate of appearance of Y-fluorescent body in interphase nuclei of normal human buccal smears, 50 coded slides (25 males and 25 females) were obtained from the medical student volunteers, stained with either one of the 3 staining solutions: quinacrine mustard buffer;quinacrine dihydrochloride water and quinacrine dihydrochloride buffer. The stained slides were blindly scorered with a count of 100 nuclei.

Although four slides were found to be misclassified, generally prediction of the sex of the individual from whom the specimen was taken was satisfactory. The mistake in classification took place mainly because of poor technical quality of the smears, which could be predicted even before decoding process. According to our experience this technique seems to be very useful in sex determination of the interphase nuclei, being complementary to the conventional sex chromatin body study.

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>國文抄錄<

口腔粘膜細胞의 休止期核에서의 Y 螢光體에 關한 硏究

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1968年 Caspersson 等이 quinacrine을 利用한 螢光染色法을 開發한 후로, 이 方法은 休止期 細胞核의 Y 螢光體와 分裂期 染色體의 帶狀構造를 硏究하는데 많이 應用되고 있다. 특히 그 가운데서도 口腔 粘膜 上皮細胞나 末稍 血液 白血球의 塗抹標本에서 Y螢光體를 檢査하여 男女의 性을 鑑別하는 方法은 벌써 臨床的으로도 많이 應用되고 있다. 그러나 우리나라에서는 아직도 이 方面의 硏究가 거의 되어있지 않아, Y螢光體에 關한 文獻上의 報告는 전혀 없는 實情이다.

이에 著者들은 正常韓國人 男女에 있어서 Y螢光體의 出現頻度를 알기 위하여, 醫大生들가운데 自願者를 選定하여, 口腔粘膜塗抹標本上의 Y螢光體 出現率을 調査하였다. 自願者들로부터 50個의 塗抹標本(男子 25個, 女子 25個)를 만들어 暗番號를 매기고, 이를 觀察者가 미리 알 수 없도록 하여 quinacrine mustard buffer, quinacrine dihydrochloride buffer 및 quinacrine dihydrochloride water 等 3가지 溶液으로 染色하여 螢光顯微鏡下에서 Y螢光體를 觀察하였다.

觀察 結果 50個의 標本가운데 男女性別의 分類가 잘못된 例가 4例 있었는데, 이 例들은 모두 技術的인 隘路때문에 抹塗 標本의 質이 좋지 못하여 觀察 當時에 벌써 失敗 例이었음을 알 수 있었다. 이들 4例를 除外하면 大體로 觀察 結果는 良好하여서 男性細胞에서는 Y螢光體의 出現率이 36%에서 78%이었고, 女性細胞에서는 4%에서 21%이어서 그 差異는 統計學的으로나 直觀的으로 보아서도 顯著하였다.

以上의 結果로 미루어 보아서 口腔 粘膜細胞의 Y螢光體 檢出方法은 性別判定에 있어서 性染色質 檢査 方法과 더불어 매우 有用한 方法임을 알 수 있었다.

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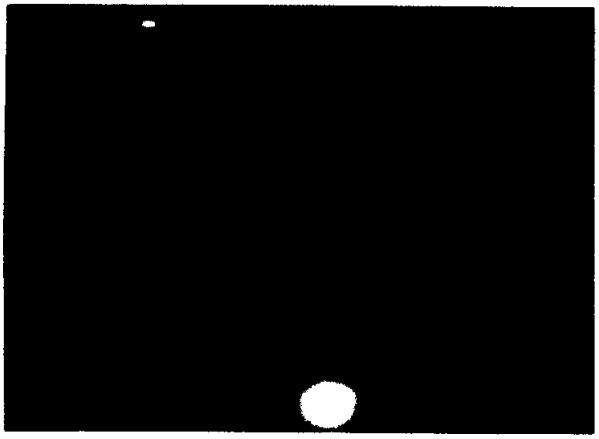

Fig. 2. Typical fluorescent spot in buccal mucosal cells of normal male (×400, orange barrier filter)

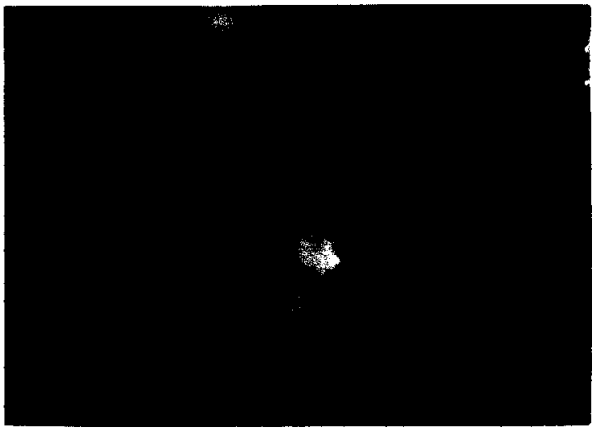

Fig. 3. Difference in interphase nuclei, between Y-fluorescence (+) cell and Y(−) Cell, (×400, orange barrier filter)

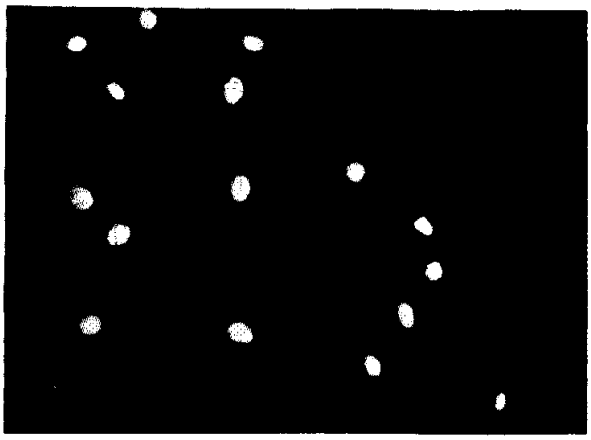

Fig. 4. Y-flurorescence could not be identified at ×100 magnification (×100, yellow-green filter.)

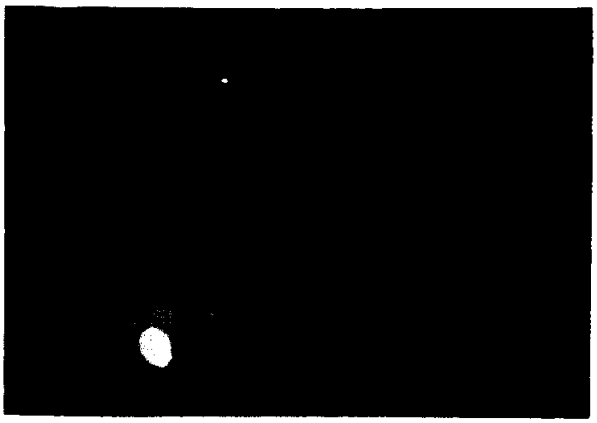

Fig. 5. (×400, yellow-green barrier filter)