Ospedale S. Camillo, Roma, Italy

Divisione Medica A. Bassi
(Head: Prof. Carlo Barbarossa)

Divisione di Medicina Pediatrica
(Head: Prof. R. Scapaticci)

EVIDENTIATION OF THE MALE CHROMATIN BODY IN HUMAN PERIPHERAL BLOOD LEUKOCYTES

A Quick Method for the Identification of Genetic Sex

G. MOSCETTI, C. ALÙ, G. ROCCHI, C.G. BARBAROSSA

SUMMARY

A survey has been carried out (using Quinacrine stain) on the frequency and position of an intensely fluorescent body (F-body) in the nuclei of leukocytes from peripheral blood smears in normal individuals and in the main syndromes from numerical alteration of sexual chromosomes.

By way of various fluorescent staining methods it is possible to easily recognize, in the human metaphases from peripheral blood cultures, the Y-chromosome for the intense fluorescence emanated by the distal part of its long arm (Zech 1969, Vosa 1970, Moscetti et al. 1971).

Thanks to this elective affinity for some acridine derivatives, it has been possible to identify the Y-chromosome in interphase human nuclei. In fact, an intensely fluorescent body (F-body), corresponding to the distal part of the long arm of the Y-chromosome, is present in the nuclei of the oral mucosa of normal males and in cultured fibroblasts and lymphocytes (Pearson et al. 1970, Moscetti and Mastroianni 1971, Majewski et al. 1971). It has further been possible to evidence the F-body in human spermatozoa (Barlow and Vosa 1970) and in cells deriving from the amniotic fluid of male foetuses (Caspersson et al. 1970).

We have endeavoured to verify the frequency of the male chromatin body in peripheral-blood polymorphonucleates and lymphocytes and its position inside the nuclei of these cells.

MATERIALS AND METHODS

Freshly taken blood smears have been fixed for 5' in 100% methanol and air dried. They have then been stained at room temperature, according to the procedure described by Pearson et al. (1970), the only difference being that we have used Medicinal Quinacrine Dihydrochloride (Atabrine, Winthrop Chemical Company, New York): ten 0.1 g tablets

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are dissolved in 100 ml of distilled water. This solution is accurately filtered before use. The observations have been made by a Zeiss fluorescence photomicroscope. The light source was a mercury vapor lamp HBO200; the excitor filter was the BG 12 and the barrier filter a 53/44 combination; the films have been obtained with Ilford HP4 and the prints with Agfa Gevaert Brovira 6, a high-contrast photographic paper.

RESULTS

Table I reports the average percentage values obtained by Conen et al. (1971) and Majewski et al. (1971), referring to the presence of the F-body in lymphocytes and polymorphs from peripheral blood smears of male and female subjects, using Quinacrine Dihydrochloride.

The table shows very high percentages of positivity of the F-body in both types of cells. The only percentage value concerning polymorphs reported by Conen et al. (1971)

TABLE I
INCIDENCE OF THE F-BODY (QUINACRINE DIHYDROCHLORIDE STAINING)

	Lymphocytes				Polymorphs			
Conen et al. 1971	34 M		17 F		20 M		12 F	
	n = 740	80.1%	n = 365	4.5%	n = 448	16.5%	n = 254	0.4%
Majewski et al. 1971	10 M		5 F		10 M		5 F	
	n = 1000	98.4%	n = 500	4.2%	n = 1000	97.3%	n = 500	2.6%

n = number of nuclei examined for each cell type.

is very modest. However the same author, with an addendum in the same work, corrected and elevated it to 60%. Furthermore, examining 613 nuclei from 33 normal males he stated that the fluorescent body was centrally located in 29.3% of the cases, peripherally located in 37% of the cases, and had a double structure in 2.3% of the cases.

Polani and Mutton (1971) have reported different percentages of positivity of the F-body in the nuclei of lymphocytes and polymorphs depending on whether the staining was done with Quinacrine Mustard Hydrochloride (Q.M.) or Quinacrine Hydrochloride (Q.) in water or in buffer. The results obtained by them are reported in Table II.

Dallapiccola (1971) refers that the proportion of cells showing the F-body in peripheral blood varies in different individuals and in different preparations between 15% and 90%.

Ricci et al. (1970) had noticed some simil-drumstick nuclear layers of about 1 mμ diameter in 25% of polymorphs of the peripheral blood of 4 members of the same family

TABLE II
INCIDENCE OF THE F-BODY ACCORDING TO STAINING TECHNIQUE (PERCENTAGE VALUES)

		Q.M.		Q. in Water		Q. in Buffer	
		M	F	M	F	M	F
Polani and Mutton 1971	Lymphocytes	69	5	44	2	58	2
	Polymorphs	37	7	35	4	14	2

Q. M. = Quinacrine Mustard Hydrochloride; Q. = Quinacrine Hydrochloride.

carrying a long Y-chromosome. By way of the Quinacrine Hydrochloride stain they evidenced the Y-body in 90% of the cells examined. Such body was situated inside the nuclei as well as in the sessile nodules and in the small drumsticks described in the males of this family.

We have used peripheral blood smears of 23 males and of 9 female chromosomically normal subjects. The percentages found with the described technique vary from 12% to 68% for lymphocites (Fig. 1), with an average value around 46%, examining 300 nuclei for each individual and for each type of cell; in polymorphs (Figs. 2-3) these percentages vary from 8% to 42% with an average value of about 30%. A fluorescent body showing a double structure was present in 1% of the cells observed and it was possible to find it only exceptionally in monocytes. In female

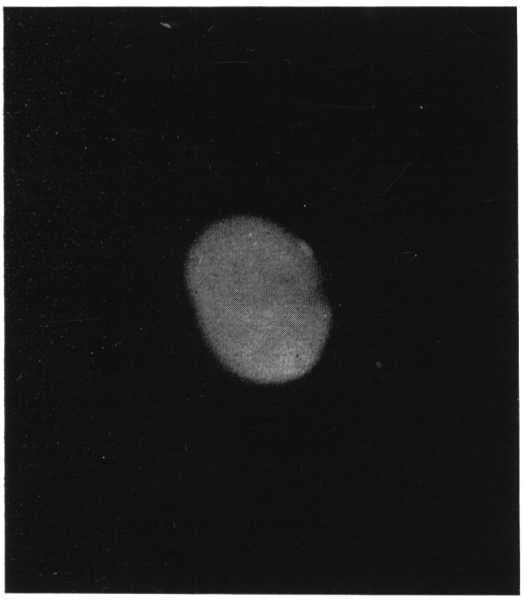
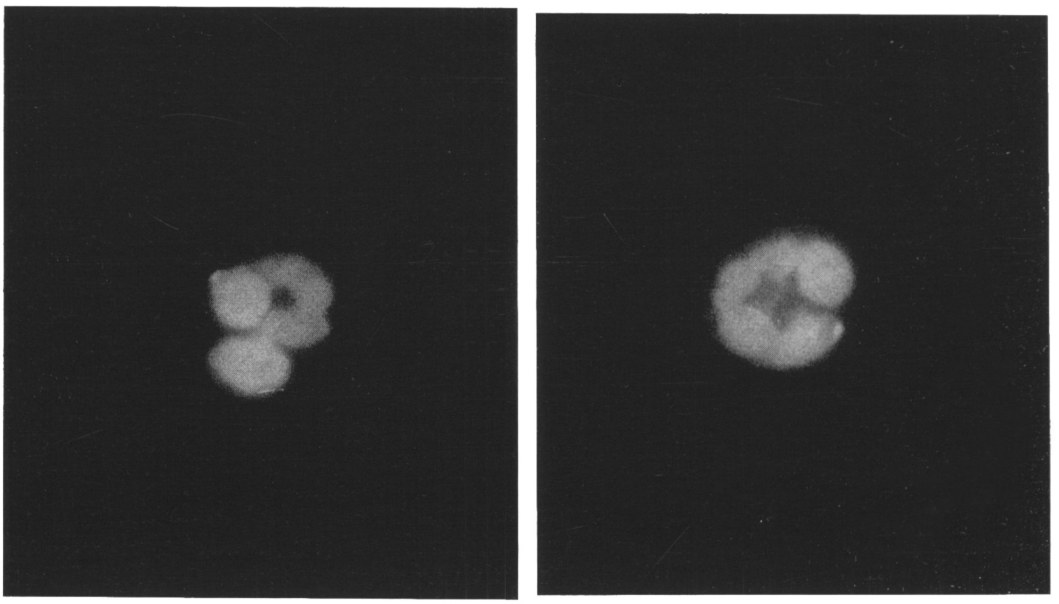


FIG. 1. *The F-body is evident in the peripheral part of the nucleus of a lymphocyte from a peripheral blood smear of a normal male.*



FIGS. 2-3. *The F-body is visible in the peripheral part of the nucleus of polymorphs from a peripheral blood smear of a normal male.*

subjects, positivity percentages were extremely low and often of dubious interpretation. In nuclear layers of male-subjects polymorphs we have evidenced the F-body with a frequency from 8% to 24% with average values around 13%. These results are close to those obtained by Lamborot-Manzur et al. (1971). Furthermore, our observations show that the F-body holds with more frequency a peripheral position in the nucleus leaning against the nuclear membrane.

In any case, from the data reported by the literature the importance of the fluorescent stain and of the technique used is evident.

We have made, among others, several observations by way of Acranil fluorescent stain (Moscetti and Mastroianni 1971, Majewski et al. 1971), but the results, which had been excellent for the nuclei deriving from epithelial cells (amniotic fluid and oral mucosa), have not proved better than others for what concerns peripheral blood smears. The easy identification of the F-body in peripheral blood smears and the simple and quick way of execution of this method, together with the research of the Barr-body in oral mucosa cells, permit a rapid and precise screening of numerical anomalies of the Y-chromosome and a valid help in the study of intersexuality problems. It is also a valid method for the research of subjects carrying XYY chromosomes: in fact, two fluorescent bodies have been observed in peripheral-blood lymphocytes and polymorphs of these subjects (Conen 1971, Thuline 1971).

The possibility to associate the research for the male F-body in peripheral blood

TABLE III
F-BODY AND GONOSOMIC COMPOSITION

Phenotype	Barr-body	F-body	Drumsticks	Caryotype	Clinical diagnosis
F	—	—	—	45,X	Turner syndrome
F	+	—	+	46,XX	Normal
F	—	+	—	46,XY	Testicular femalization syndrome
	+				
F	++	—	++	47,XXX	Superfemale
M	—	+	—	46,XY	Normal
M	+	+	+	47,XXY	Klinefelter syndrome
	+		+		
M	++	+	++	48,XXXY	Klinefelter XXXY syndrome
	+		+		
	++		++		
M	+++	+	+++	49,XXXXY	Klinefelter XXXXY syndrome
M	+	++	+	48,XXYY	Klinefelter XXYY syndrome
M	+	—	+	46,XX	Adrenogenital syndrome
M	—	++	—	47,XYY	XYY syndrome
M	+	—	+	46,XX	Prenatal hormonal masculinization syndrome

smears with that for chromatin in oral smears, permits a rapid identification of anomalies of number in the gonosomes. Moreover, in some cases we have been able to identify the XXY constitution through the only examination of polymorphs of peripheral blood smears and the contemporary finding in them of drumsticks and F-bodies.

In this way, it has been possible for us to establish correctly the gonosomic composition (later confirmed by the caryotype examination) in several of the syndromes reported in Table III.

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Riassunto

È stata condotta una ricerca (usando la Quinacrine come colorante) sulla frequenza e la posizione di un corpo intensamente fluorescente (*F-body*) nei nuclei di leucociti di sangue periferico sia in individui normali che nelle principali sindromi con alterazione numerica dei gonosomi.

Résumé

Une recherche a été conduite (moyennant une coloration à la Quinacrine) sur la fréquence et la position d'un corps intensément fluorescent (*F-body*) dans le noyau de leucocytes de sang périphérique chez des individus normaux ainsi que dans les principaux syndromes avec altération numérique des gonosomes.

Zusammenfassung

Mit Hilfe von Quinacrine als Farbstoff untersuchten wir das Vorkommen und die Position eines stark fluoreszenten Körpers (*F-body*) in den Leukozytenkernen peripheren Bluts bei normalen Individuen sowie bei den wichtigsten mit Alteration der Gonosomen verbundenen Syndromen.

Dr. Giacomo Moscetti, Ospedale S. Camillo, Divisione Medica A. Bassi, Circ.ne Gianicolense 87, 00152 Roma, Italy.