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G-BANDING OF PACHYTENE BIVALENTS IN HUMAN SPERMATOCYTES

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SUMMARY

G-banding of meiotic pachytene chromosomes is obtained using trypsine procedure. G-bands are sufficiently pronounced to allow identification of several bivalents. Our results show the equivalence of the G-band patterns between mitotic chromosomes and meiotic bivalents. The G-band patterns of mitotic-meiotic chromosomes also correlate closely with chromomere patterns of meiotic pachytene bivalents.

For several years, the identification of pachytene bivalents in human male meiosis has aroused the interest of cytogeneticists.

The difficulty in obtaining 23 dispersed bivalents explains the paucity of results. Analysis of chromomere patterns normally visible along pachytene bivalents has led to the identification of acrocentric bivalents 13, 14, 15, 21 and 22 (HUNGERFORD *et al.*, 1971 *a* and *b*) and bivalents 10 and 11 (FERGUSON-SMITH and PAGE, 1973). Another bivalent, n° 9, has been identified thanks to its structural (presence of parameres : HUNGERFORD *et al.*, 1972) and staining properties (Giemsa stain at pH 11.6 : GAGNE *et al.*, 1973 ; PAGE, 1973).

Banding techniques have been too little used in studying meiotic chromosomes. The ARRIGHI and HSU technique (1971) permits one to obtain C-bands, however these are often present in the absence of any denaturing treatment (LUCIANI *et al.*, 1972 and fig. 1). Quinacrine mustard staining (CASPERSSON *et al.*, 1971) allows the identification of bivalents during diakinesis, yet this method has not been applied to the analysis of pachytene bivalents.

The use of trypsin digestion permits one to obtain G-bands along pachytene bivalents. This method, plus a modification of technique permitting bivalent dispersion, leads to the identification of certain bivalents.

FIG. 1. — *Pachytene nucleus showing 23 dispersed bivalents with prolonged hypotonic treatment*
Only constitutive heterochromatin is visible (arrows)

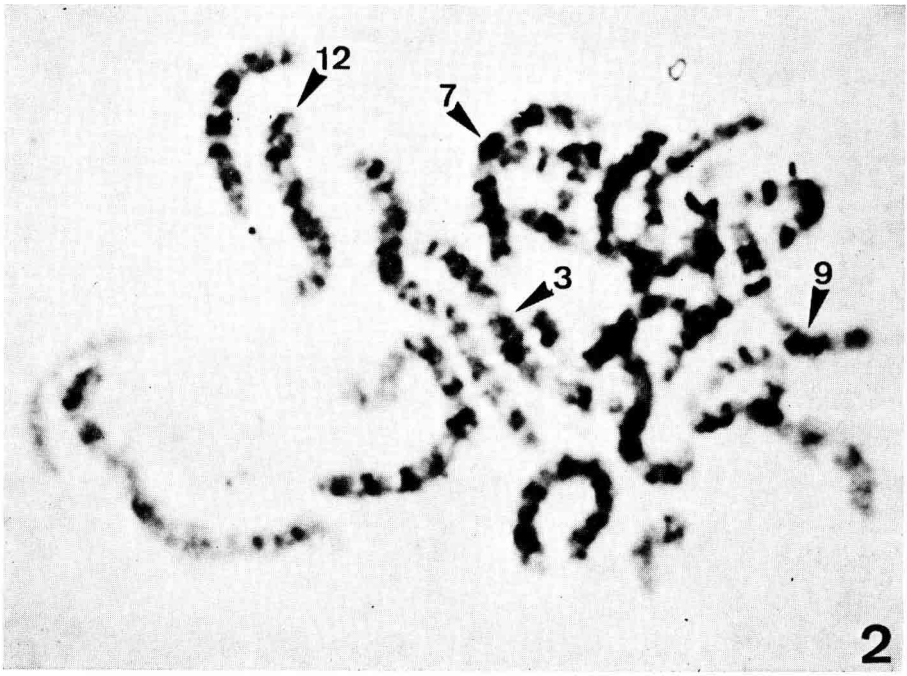

FIG. 2. — *G-banding patterns revealed along pachytene bivalents with trypsine digestion*
Bivalents 3, 7, 9 and 12 can be easily identified

MATERIAL AND METHODS

Meiotic preparations were obtained from two subjects examined for sterility. A testicular fragment obtained by biopsy, was immediatly immersed in a 0.44 p. 100 hypotonic potassium chloride (LUCIANI *et al.*, 1971). The hypotonic treatment was prolonged from 8 to 10 hours. The sample was then transfered and dilacerated in a methanol and glacial acetic acid solution (3 vol/1 vol.). The resulting cellular suspension was centrifuged at 800 r.p.m. for 7 minutes. The pellet was resuspended in 5 mls of 45 p. 100 acetic acid, centrifuged once again and the final suspension was spread on pre-cooled slides.

Slides thus obtained were placed in 2 × SSC solution at 40°C for 2 hours, then treated with a 0.25 p. 100 trypsin solution for 10 seconds at 18°C and finally stained in Giemsa solution.

RESULTS AND DISCUSSION

Under trypsin action G-bands appear along the pachytene bivalents (fig. 2). Sequence analysis of the bands on isolated bivalents shows an exact correspondance with G-bands characteristic of mitotic chromosomes. Thus, it is possible to identify with certitude certain bivalents (fig. 3 and 4).

Our results show the existance of an equivalence between G-bands of meiotic and mitotic chromosomes. In addition, the equivalence exists between G-banding

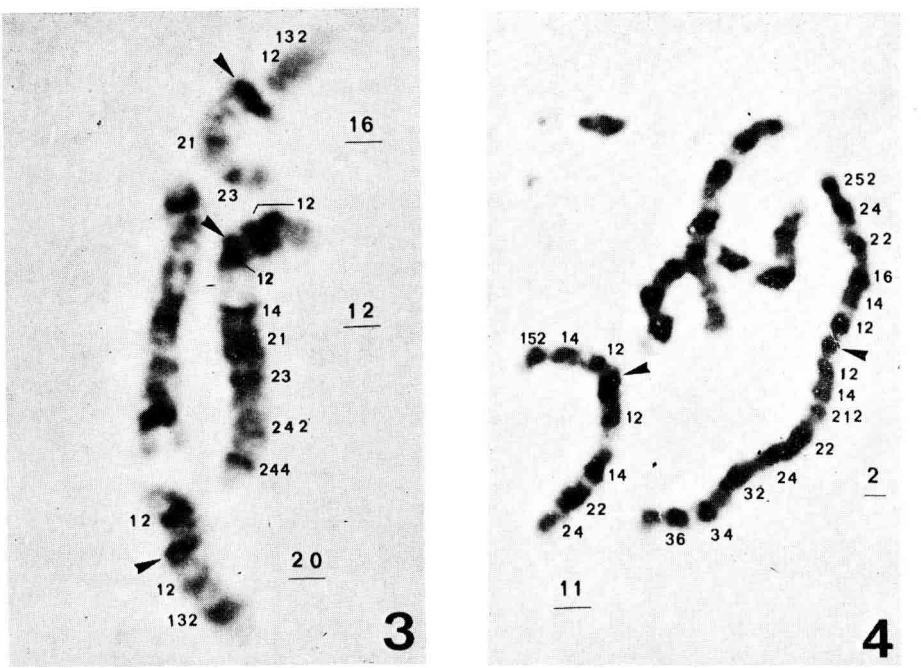

FIG. 3 and 4. — *Isolated bivalents identified by their G-banding patterns*

FIG. 3. — *Bivalents 12-16 and 20*

FIG. 4. — *Bivalents 2 and 11.* Arrows indicate the centromere

patterns of meiotic or mitotic chromosomes and the chromomere sequence described by HUNGERFORD *et al.*, for acrocentric bivalents (1971 *a*, *b*) and by FERGUSON-SMITH and PAGE for bivalents 10 and 11 (1973). Finally, the reproductibility of the method should rapidly lead to the mapping of pachytene bivalents.

Colloque D. G. R. S. T., Biologie de la Procréation, Paris, 7-8 mars 1975.

RÉSUMÉ

IDENTIFICATION DES BIVALENTS DU STADE PACHYTÈNE DANS LE SPERMATOCYTE HUMAIN PAR LA TECHNIQUE DES BANDES G (TRYPSINE)

Sur les chromosomes du stade pachytène l'utilisation de la trypsine permet d'obtenir des bandes G suffisamment nettes pour permettre l'identification de plusieurs bivalents. Nos résultats montrent l'équivalence des bandes G entre les chromosomes mitotiques et les bivalents méiotiques.

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