

## **Micro-flow fluorometric DNA measurements of isolated testicular cells from the rat**

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**Summary.** Suspensions of testicular cells from 17 to 150-day old rats were stained with a fluorochrome (ethidium bromide) and subjected to micro-flow fluorometry (MFF). The resulting DNA distribution patterns consisted of two to three peaks of fluorescence, the relative size of which altered with increasing age. Up to day 25, two peaks of fluorescence were detected. One peak represented diploid cells (2 C) and the other tetraploid cells (4 C). Following an accumulation of cells in S phase and of tetraploid cells beginning around day 20, a third peak of fluorescence representing a haploid cell population (1 C) became apparent from suspensions of animals 27 days of age. In mature rats the haploid cells constituted about 75 p. 100 of the fluorescing testicular cells. It is also shown that treatment with oestradiol-benzoate prevents the completion of meiosis in immature rats.

MFF can also be used to assess the degree of contamination with tubular cells in Leydig cell preparations obtained by collagenase treatment. It is concluded that MFF offers a practical and sensitive technique for studying selected aspects of spermatogenesis involving changes in the DNA frequency distribution pattern of testicular cells.

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### **Introduction.**

Studies on meiosis in the rat often necessitate detailed examination of numerous histological sections and require an ability to recognize specific tubular cell types. Therefore, in the field of male reproduction there is a need for methods which can provide a rapid and sensitive measurement of meiotic changes in testis tissue. By means of micro-flow fluorometry (MFF) (van Dilla *et al.*, 1969 ; Göhde and Dittrich, 1971) large numbers of cells in suspension can be measured in a short time and ranged according to their DNA content. Since the various steps of proliferation and transformation in the process of spermatogenesis involves distinct changes in the DNA content of the germ cells, this technique offers the possibility of monitoring such changes with relative ease.

The aim of the present study was to use MFF to monitor the development of meiosis from dispersed testicular cells in immature rats and to investigate hormonal long term effects on the completion of meiosis.

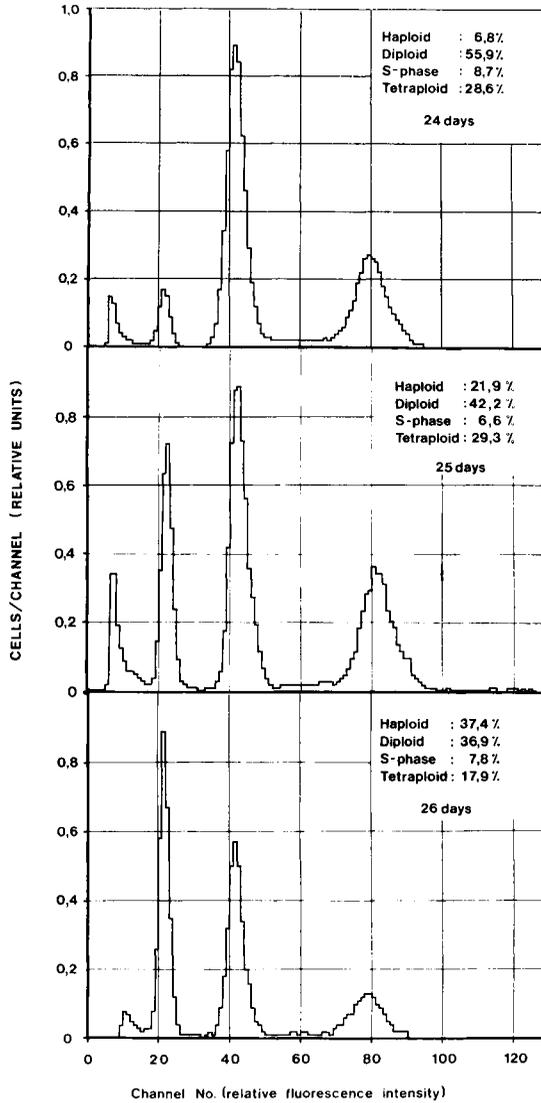


FIG. 1. — DNA distribution histograms of isolated testicular cells from immature rats measured by micro-flow fluorometry. The cells were isolated by means of collagenase treatment, fixed in absolute ethanol, RNase and pepsin treated before they were stained with the fluorochrome ethidium bromide and measured. The abscissa shows the relative fluorescence intensity reflecting the cellular DNA content and the ordinate the relative cell numbers. The pulses to the left of channel 20 represent cell debris and non specific fluorescence. Three normally distributed peaks of fluorescence are observed. The first peak located around channel 20 represents haploid cells, the second peak around channel 40 diploid cells and the peak around channel 80 tetraploid cells. Note the increase in the proportion of haploid cells and the parallel decrease in the proportion of tetraploid cells from day 24 to day 26.

Since haploid cells constitute 75 p. 100 of the fluorescing cells in the mature testis, the relative amount of haploid cells in Leydig cell enriched preparations obtained by collagenase treatment has been used as an indicator of tubular contamination and therefore as an indirect way of assessing the purity of these cell preparations.

### Materials and methods.

Isolated testicular cells were obtained by mincing decapsulated testes into small pieces followed by collagenase incubation of the resulting testis material. Single cell suspensions were fixed in absolute ethanol, stained with the fluorochrome ethidium bromide and measured by a Pulse Cytophotometer (Phywe AG, Göttingen, W. Germany). For details about the cell preparation and MFF measurements, see Clausen *et al.* (1977). From day 7 after birth, groups of Sprague-Dawley rats were injected daily with 0.1 ml oil i.m., with 2.5 mg DHTP i.m. plus 50  $\mu\text{g}$  FSH s.c. in 0.1 ml oil, and with 5  $\mu\text{g}$  oestradiol ( $\text{E}_2$ ) in 0.1 ml oil i.m. From these groups and from untreated animals, testicular cells were isolated at 2-day intervals from day 17 to day 38 after birth. From the untreated animals, cells were isolated up to day 150. Testicular cells were also isolated from hypophysectomized rats and from rats with the Tfm (testicular feminized male) syndrome.

For preparation of interstitial cells from 60-day old rats the procedure of Moyle and Ramachandran (1973) was followed with little modification.

### Results and discussion.

In smears from testes from animals of various ages the numbers of typical Leydig cells were approximately 2 p. 100 of the total number of nucleated cells present. Moreover, the numbers of connective tissue cells (fibrocytes, fibroblasts, vascular cells, macrophages) and nucleated blood cells (leucocytes, lymphocytes) in the same preparations were negligible. Cells within a certain DNA class could be detected when they constituted more than 0.2 p. 100 of the total cell population. It should be noted that pepsin treatment (Berkhan, 1972) removes the cellular cytoplasm, so that all measurements refer to single nuclei.

Figure 1 shows typical DNA histograms of dispersed testicular cells derived from young rats of different ages. The peak close to channel 20 represents haploid cells (1 C), the peak close to channel 40 diploid cells (2 C), and the last peak located around channel 80 tetraploid cells (4 C). The pulses to the left of the haploid peak represent non-specific fluorescence and fluorescence of cell debris. The histograms in figure 1 illustrate the increasing number of haploid cells with increasing age of immature rats. Age-related changes in the proportion of haploid cells, tetraploid cells and cells with S phase DNA content are also shown in figure 2. Prior to the appearance of haploid cells in immature rats there is a temporary and shortlasting increase in the proportion of cells with S phase DNA content, and a temporary but longer-lasting increase in the proportion of tetraploid cells. The observed peaks are assumed to represent the DNA synthesizing- and the tetraploid stages, respectively, of primary spermatocytes.

It is further shown that the first detection of haploid cells varied from day 20 to day 27 after birth in three different studies, and that such differences may easily and accurately be detected by MFF.

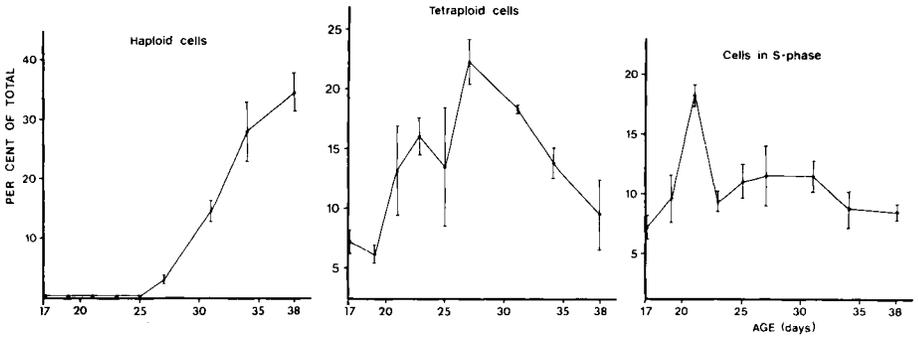


FIG. 2. — Age related changes in the proportion of haploid, tetraploid and S phase cells of isolated testicular cells from rats. Note the increase in the proportion of cells with S phase and tetraploid DNA content prior to the appearance of haploid cells, and the inverse relationship between the relative numbers of haploid and tetraploid cells after day 27.

The effects of DHT plus FSH, and of  $E_2$  on the appearance of haploid cells in young rats with increasing age are shown in figure 3. DHT plus FSH caused no significant change in the relative number of haploid cells detected, compared with

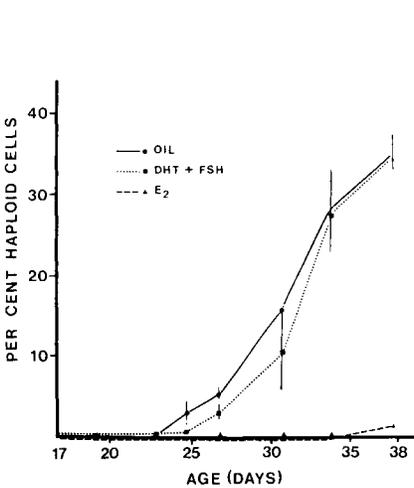


FIG. 3.

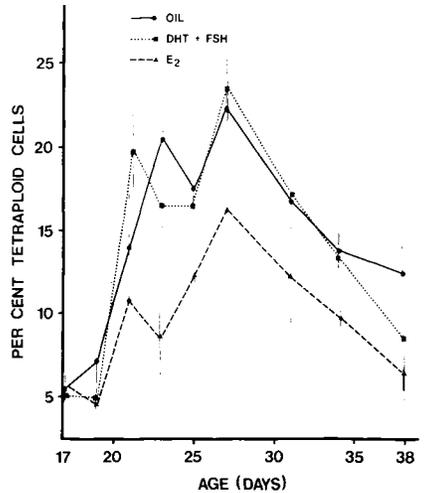


FIG. 4.

FIG. 3. — Effects of long-term administration of DHT + FSH and  $E_2$  on the completion of meiosis in male rats. Injections were given daily from day 7 after birth until the animals were killed.

FIG. 4. — Effects of long term administration of DHT + FSH and  $E_2$  on the proportion of tetraploid cells in testicular cell suspensions from rats. Injections were given daily from day 7 after birth until the animals were killed.

controls, whereas  $E_2$  suppressed the appearance of haploid cells totally until day 38. Figure 4 shows that DHT plus FSH did not significantly influence the occurrence of the peak of tetraploid cells, whereas  $E_2$  resulted in a reduction in the proportion of tetraploid cells.

In the adult testis about 75 p. 100 of the fluorescing cells were haploid and about 5 p. 100 tetraploid. Characteristic DNA frequency distributions of testicular cells were obtained from hypophysectomized rats and from rats of the Tfm syndrome, both exhibiting reduced numbers of haploid cells compared with normal littermates. This indicates an interruption, but no abolishment of the reduction division. The marked increase in number of tetraploid cells in the Tfm syndrome suggests a partial block of spermatogenesis at the transition of primary to secondary spermatocytes.

When enriched Leydig cell suspensions were subjected to MFF, the relative amount of haploid cells in these suspensions was a good indicator of the degree of tubular contamination. By using a cell suspension of whole testis from animals in the same group as a standard, one can accurately calculate the degree of tubular contamination and therefore also the purity of these cell suspensions.

## Conclusion.

The present study clearly shows that selected aspects of those processes of spermatogenesis which involve changes in DNA frequency distribution can be accurately studied by MFF of dispersed testicular cells. It is demonstrated how hormones can prevent the completion of meiosis, and suggested that MFF may be used to assess the purity of Leydig cell enriched suspensions.

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**Résumé.** Des cellules testiculaires en suspension, de rats âgés de 17 à 150 jours, ont été colorées par un fluorochrome (ethidium bromide) et analysées par « micro-flow » fluorimétrie (MFF). Les histogrammes des valeurs de l'ADN consistaient en deux à trois pics de fluorescence dont la hauteur relative changeait avec l'âge des animaux.

Jusqu'au 25<sup>e</sup> jour, deux sommets de fluorescence ont été enregistrés. L'un représentait les cellules diploïdes (2 C), l'autre les cellules tétraploïdes (4 C). Après une augmentation des cellules en phase de synthèse de l'ADN et des cellules tétraploïdes débutant vers le 20<sup>e</sup> jour, un troisième sommet de fluorescence, représentant les cellules haploïdes (1 C) apparaissaient dans les suspensions cellulaires des animaux âgés de 27 jours.

Chez les rats matures les cellules haploïdes représentent près de 75 p. 100 des cellules testiculaires fluorescentes.

On constate aussi que le traitement par le benzoate d'œstradiol empêche la méiose chez les rats immatures. Le MFF peut aussi être utilisé pour déterminer le degré de contamination par des cellules tubulaires dans les suspensions de cellules de Leydig obtenues par le traitement par le collagénase.

Le MFF offre un moyen pratique et sensible pour l'étude des aspects précis de la spermatogenèse où le changement du contenu d'ADN des cellules testiculaires est en jeu.

### References

- BERKHAN E., 1972. DNS-Messung von Zellen aus Vaginal-abstrichen. *Arzt. Lab.*, **18**, 77-79.
- CLAUSEN O. P. F., PURVIS K., HANSSON V., 1977. Application of micro-flow fluorometry to studies on meiosis in the male rat. *Biol. Reprod.*, **17**, 555-560.
- GÖHDE W., and DITTRICH W., 1971. Impulsfluorometrie, —ein neuartiges Durchflussverfahren zur ultraschnellen Mengenbestimmung von Zellinhaltsstoffen. *Acta histochem (Jena) Suppl.* **10**, 429-437.
- MOYLE W. R., RAMACHANDRAN J., 1973. Effect of LH on steroidogenesis and cyclic AMP accumulation in rat Leydig cell preparations and mouse tumor Leydig cells. *Endocrinology*, **93**, 127-134.
- VAN DILLA M. S., TRUJILLO T. T., MULLANEY P. F., COULTER J. R., 1969. Cell microfluorometry : a method for rapid fluorescence measurement. *Science*, **163**, 1213.
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