CARYOMETRIC STUDY OF SPERMATOGENESIS IN THE RAT

A. L. FERREIRA, L. LISON and V. VALERI

Department of Functional and Applied Human Morphology
Ribeirão Prêto School of Medicine
Universidad de São Paulo

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Summary. The nuclear volume of the cells of the seminiferous epithelium was studied throughout the stage of the spermatogenesis in the rat, and the moment of the pre-meiotic synthesis of DNA was determined through quantitative histophotometric measurements.

During spermatogenesis the cells showed a sudden volume variation from Spermatogonia A until the initial period of stage Pachytene. Next, followed a long period of continuous growth of the nuclear volume, ending at the first meiotic division.

The premeiotic synthesis of the DNA was represented by a duplication of the amount of DNA of the nucleus; this happened during the stage Spermatocyte I “resting”. It was not followed by a duplication of the nuclear volume, but by an increase of only approximately 47%.

Our data suggest that the sudden modifications in the nuclear volume during the period between the stages Spermatogonia A and Zygotene + Pachytene A, are caused by asynchronous duplication or demediation of two different types of nuclear constituents.

The continuous growth of the nuclear volume would depend upon a different mechanism.

1) During spermatogenesis the cells show sudden volume variation from Spermatogonia A until the initial period of stage Pachytene, Pachytene A according to our nomenclature. Taking the nuclear volume during the stages Zygotene and Pachytene A for reference and assuming 2.000 as their arbitrary value, during this period of spermatogenesis the mean nuclear volume evolves as follows:

   Spermatogonia A: 2.192; Spermatogonia I: 1.532 Spermatogonia B and “Resting A”: 0.957; “Resting B” and Leptotene: 1.407; Zygotene and Pachytene A: 2.000. It is assumed that during this period of the spermatogenesis there are demediations of nuclear volume followed by a return to its initial volume; however, in the latter as well as in the former there are intermediary phases which constitute Schröder’s “sesquiphases” (1947).

2) Next, follows a long period of continuous growth of the nuclear volume, ending at the first meiotic division. The maximum value observed corresponds to 8.570 in our system of units.

3) The volume of the spermatocyte II is not constant; throughout its existence it increases from a initial value which could not be exactly determined till a volume corresponding to a 2.582 value. This value is well below half of the Spermatocyte I in stage Diplotene.

   The volume of the spermatid does not change during the initial period of its existence. Its mean value equals 2.146, which is 1/4 of that of the Spermatocyte I in stage Diplotene. After this initial period the Spermatid volume decreases; for technical reasons measurements were not conducted during this period.

4) It was found that the premeiotic synthesis of the DNA is represented by a duplication of the amount of DNA of the nucleus; this happens during the stage Spermatocyte I “resting”. “Resting A” is the stage before the duplication of the DNA.

5) The duplication of the amount of the DNA, in stage Spermatocyte I “resting”, is not followed by a duplication of the nuclear volume, but by an increase of only approximately 47%.

6) Our data suggest that the modifications in the nuclear volume during the period between the stages Spermatogonia A and Zygotene + Pachytene A, are caused by asynchronous duplication or demediation of two different types of nuclear constituents: the first shows a variation proportional to the amount of the nuclear DNA while the variation of the second is not related to the DNA; the latter is called “residual material” in order to avoid any assumptions about its nature.

It was possible to calculate the relative volumes related to these two types of constituents. Being 2N the volume related to the diploid amount of DNA and 2R the volume related to the
"residual material" found in the stage Zygotene + Pachytene A, the following values were calculated, with our system of units: $2N = 0.429; 2R = 1.108$.

7) The observed variations in volume are in agreement with the hypothesis which assumes a variation of the constituents according to the following scheme:

Spermatogonia I: $2N + 2R$; Spermatogonia B and "Resting A": $2N + 1R$; "Resting B" and Leptotene: $4N + 1R$; Zygotene and Pachytene A: $4N + 2R$.

8) The period of the continuous growth of the nuclear volume from stage Pachytene B till stage Diplotene, depends upon a different mechanism, as the "residual material" does not vary in simple definite proportions.

9) During the first meiotic division the "residual material" seems to pass into the cytoplasm. It returns, only partially, to the nucleus during the life-span of Spermatocyte II. After the second meiotic division, the "residual material" returns completely to the nucleus of the Spermatids; therefore, the whole four daughter spermatids, originating from one Spermatocyte I, contain exactly the same amount of "residual material" found in Spermatocyte I, immediately before its first meiotic division.

Introduction

Only a few papers are concerned with the study of the variations in the nuclear volume during spermatogenesis. In invertebrate animals studies were performed only on insects: *Bombyx mori* (Wermel, 1933); *Arelius albopunctatus* (Schrader and Leuchtenberger, 1950); in some Triatomides (Schreiber and Pellegrino, 1951). In non-mamall, vertebrate animals, there is a reliable study by Schreiber (1947) on several ophidians and the work of Kretschmann (1955) on *Bufo*. In mammals, research studies were carried in the rat (Hertwig, 1931); Roosen-Runge (1955), Merkle (1957), in the mouse (Hertwig, 1931), in the guinea-pig (Jacob, 1926), in the cat (Hertwig, 1933) and in man (Hertwig, 1933). Although valuable, these investigations in general show one, sometimes both of the following limitations:

1. Only a small number of stages of the spermatogenesis was identified and taken into consideration. In the present investigation three types of spermatogonia, seven stages in the evolution of spermatocytes I, two of spermatocytes II, and one stage of spermatid were identified in a sequence which comprises 32 successive steps.

2. Unsatisfactory statistical treatment in the evaluation of the results. Most works are based either on visual comparison of histograms or on curves of distribution of frequency without further statistical elaboration.

The nuclear amount of DNA seems to be one of the factors which determine the nuclear volume. In the papers mentioned above no comparison between nuclear volume and amount of DNA was made. In the present paper the nuclear volumes were measured and the DNA assayed histophotometrically. A correlation between these two measurements was established.

Material and Methods

1. Material and Histological Technique

The present research was performed on histological sections of the testis of an healthy adult Wistar rat.

The testis, removed under general anaesthesia, was fixed in a mixture of alcohol (85 ml), formaldehyde (10 ml) and acetic acid (5 ml), during 24 hours embedded in paraffin and 15 and