The Chromosomes of the Great Indian Rhinoceros (*Rhinoceros unicornis* L.)

A skin biopsy was obtained from a female great Indian rhinoceros (*Rhinoceros unicornis* L.) at the National Zoological Park in Washington, D.C. The specimen was cultured in Eagle's basal medium with 10% calf serum. After subculture in Carrel flasks, the cells were treated with a hypotonic solution of Earle's balanced salt solution (1:3), fixed in acetic acid-methanol fixative (1:3), and air-dried on slides. Some cultures were treated with H^3-thymidine to allow study of the replicatory pattern with special reference to the *X*-chromosomes. The details of the above procedures have been published elsewhere[1,2].

From examination of 41 adequately spread cells the diploid number was determined to be 82. 10–12 pairs of chromosomes appear to have subterminal and all the rest terminal centromeres. The autoradiographs showed one of the largest chromosomes, and the most metacentric of the complement, to be late labeling. This pair of chromosomes is therefore taken to be the *X*-chromosomes. A karyotype is shown in the Figure.

Chromosomes of *Rhinoceros unicornis*. × 1600.

The white rhinoceros (*Ceratotherium sinum* Burchell 1817) has also been found to have a diploid number of 82[3]. The karyotypes of both the species of rhinoceros appear to be similar, although the Indian rhino may possess several more subterminal and several less terminal elements than the white rhino. In *Diceros bicornis* Gray, HUNGERFORD et al.[4] have found a diploid number of 84.

The Rhinocerotids have the highest diploid chromosome number of all the species of mammals so far investigated[5].

Zusammenfassung. Die Chromosomen des indischen Rhinozeros wurden untersucht. Es wurden 82 Chromosomen gefunden, die auch in ihrer Struktur den Chromosomen des afrikanischen Breitmaulnashorns ähnlich sind.

DORIS H. WURSTER and K. BENIRSCHKE

Department of Pathology, Dartmouth Medical School, Hanover (New Hampshire 03755, USA), 22 December 1967.

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The Effect of Cigarette Smoke Inhalation on Spermatogenesis in Rats

Large scale epidemiological studies carried out recently have unanimously proved the harmful effect of smoking on health[1]. Spermatological investigations showed that prolonged heavy smoking induces a decrease in the overall number of spermia, pathogenic forms are more often encountered and smoking has an unfavourable effect on the motility of spermia too[2].

The present experiments were aimed to find out which phase of spermatogenesis is actually affected by smoking.

Method. Fourteen mature white male rats of Wistar strain weighing 160–200 g were kept for 6 weeks in a so-called smoking chamber[3]. The animals inhaled cigarette smoke for 15 min 8 times daily. During the inhalation periods nicotine concentration in the chamber was 8–23 mg/m³. After every 15 min of 'smoking' the chamber was cleared of the smoke. The animals were kept on a standard diet and were allowed to drink freely.

The treatment lasted for 6 weeks after which body weight of the rats was taken and the animals were killed by decapitation. The rats were then dissected, the testes removed, weighed and fixed in 4% formaline. Paraffin embedding followed, of which 6 μ sections were prepared and dyed with hematoxylin-eosin and azan. After a qualitative histological examination, the quantitative registration of spermatogenesis was attempted as follows:

(1) Using the method of ROOSEN-RUNGE and GIESEL (RRG) the frequency of the spermatogenetic phases was determined[4]; (2) the volume of the individual cell types corresponding to each of the RRG phases was measured by karyometry using a × 3000 projection magnification[5]; (3) young and old primary spermatocytes as well as the spermatides of each RRG phase were counted. A divided field ocular was used and only the tubuli with round lumina were counted.

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Data obtained with the above procedure were compared to the corresponding histological findings and values of 8 control rats of the same strain, age and weight.

Results. During the 6 weeks of treatment there was a 10 to −15 g change in the body weight of the animals. Dissection showed no macroscopic lesions. Weight of the testes proportioned to body weight showed no difference in the experimental and control groups.

Histological examination of the testes showed more primary spermatocytes and more abnormal mitotic forms with an enlargement of the nuclei in the experimental group.

Determination of spermatogenetic phases with the RRG method revealed a significantly increased frequency of phase IV and a considerable decrease of phase V (Figure 1).

Karyometric examination proved that whereas nuclei of the primary spermatocytes are considerably enlarged prior to mitosis, nuclear volume of spermatides is considerably reduced as compared to findings in the controls (Figure 2, Table).

Figure 3 shows mean values and standard deviations in the spermatogenetic phases. It could be proved that the number of old spermatocytes increased before mitosis, the number of spermatides was, however, significantly lower than that of the controls.

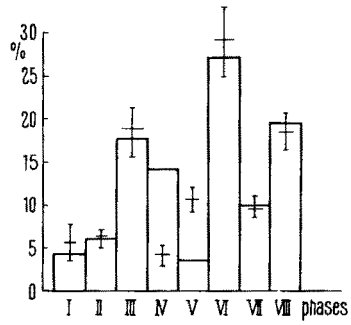

Fig. 1. Frequency of spermatogenetic phases in the testes of cigarette smoke treated and control rats. Values of controls are shown in the horizontal lines with 5% confidence limit.

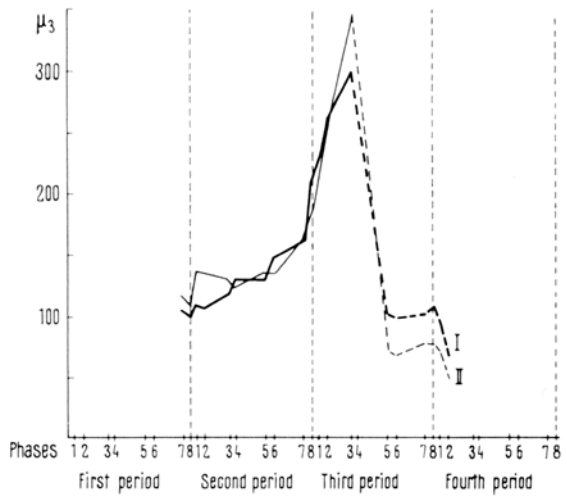

Fig. 2. Nuclear volume of spermatogenetic cells according to the RRG phases. I, control rats; II, cigarette smoke treated rats.

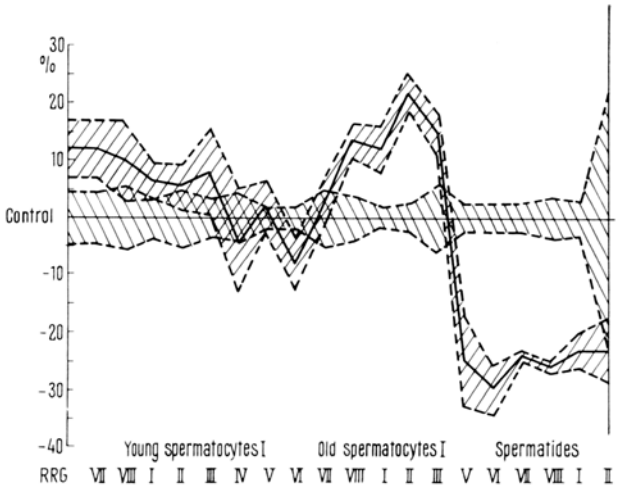

Fig. 3. Number of old and young spermatocytes and of spermatides in each of the RRG phases.

Nuclear volume of spermatogenetic cells in the testes of control and cigarette smoke treated rats

		I	II	III	IV	V	VI	VII	VIII
Young spermatocytes I	Control							106.2	100.9
	Smoking							118.8	111.1
Young spermatocytes I	Control	110.1	107.6	120.3	130.1	132.3	148.0		
	Smoking	137.5	134.9	130.4	124.3	135.3	136.7		
Old spermatocytes I	Control							163.3	209.3
	Smoking							166.0	186.4
Old spermatocytes I	Control	224.0	262.0	301.4					
	Smoking	218.0	251.0	347.0					
Spermatides	Control					102.2	100.1	103.9	107.5
	Smoking					76.6	69.8	79.3	80.0
Spermatides	Control	97.3	68.5						
	Smoking	74.7	52.8						

Discussion. The present experiments have unanimously proved that cigarette smoke inhalation induces characteristic lesions to spermatogenesis in rats. The primary spermatocytes appear to be the target of the noxa as cigarette smoke inhalation induces a swelling of the nuclei and an increase in the number of old spermatocytes prior to mitosis. But a mitotic inhibition of the spermatocytes can also be proved as an abnormal increase of RRG phase IV and a frequency of abnormal mitotic forms was found. Mitotic inhibition induces a decrease of spermatides with a reduced nuclear volume which is responsible for a low frequency of phase V.

Cigarette smoke inhalation damages the process of spermatogenesis by affecting mitosis in the spermatocytes. Noxae of spermatogenesis (e.g. ionising radiation[6]) generally exert their harmful effect mainly on primary spermatocytes. These cells seem to be the most susceptible to environmental noxae.

Several investigators have reported lesions to the testes induced by smoking or nicotine treatment[7-9], others have questioned such an effect[10-12]. Quantitative investigations into the process of spermatogenesis have, however, proved that cigarette smoke inhalation causes specific lesions in the development of spermia by inhibiting mitosis of the spermatocytes.

Zusammenfassung. Durch die Einatmung von Zigarettenrauch wurden bei Ratten Störungen der Spermiogenese beobachtet. Es kam zu einer Hemmung der Zellteilung von Spermatocyten.

M. Viczián

Department of Obstetrics and Gynaecology of the Central State Hospital, Budapest (Hungary),
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Salivary Chromosome-Like Structure of a Coccid Chromosome

Cytological researches in recent years have accumulated a wealth of information on the morphological and structural organizations of both animal and plant chromosomes[1,2]. In addition to those chromosomes which are usually seen in the mitotic and meiotic cells, there are also a few special types of chromosomes, the chief among them being (1) the lamp brush chromosomes of the amphibian oocytes, (2) the salivary gland chromosomes of the dipteran insects like *Drosophila, Chironomous, Camptomya, Sciara* and *Rhyncosciara* and (3) the accessory or supernumery or 'B' chromosomes, whose number vary from one to many.

While reports are available on the occurrence of supernumery chromosomes in Coccids[3,4], there is no reference for the occurrence of chromosomes which resemble those of the salivary gland in species such as coccids where the chromosome is holokinetic. The salivary chromosomes so far studied are all from the dipteran insects. These chromosomes are the largest ones and their importance in the field of cytogenetics has been stressed by many earlier workers[5-9]. Along with their giant size, the salivary

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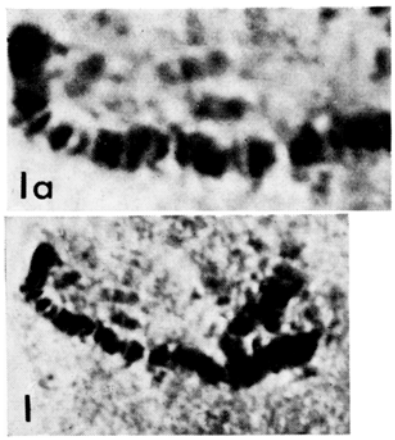

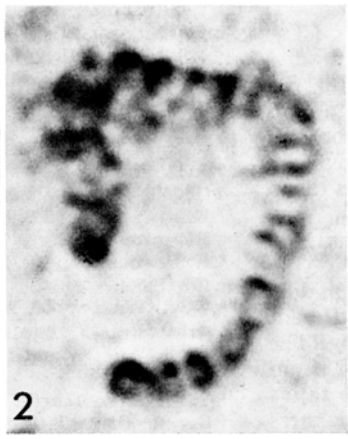

Fig. 1. The haploid compliment of 2 chromosomes from an embryo; 1 long and another short (bent) chromosome. Note the chromatic and achromatic banding pattern. Ca. × 2910.

Fig. 1a. Enlarged portion of a long chromosome. Ca. × 4365.

Fig. 2. Similar type of chromosome from a different embryo. Ca. × 4365. (All Figures are stained with Schiff's reagent.)