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Aneuploidy and polyploidy in germ-line cells of the male Chinese hamster (*Cricetulus griseus*)

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A search for aneuploidy and polyploidy in germ-line cells of the male Chinese hamster ($n=11$) gave the following results: (1) Fourteen out of 225 spermatogonial metaphases were apparently tetraploid, 4 hexaploid and 2 octaploid. (2) Three out of 174 first spermatocytes had 22 bivalents. (3) Five out of 392 second metaphases had 22 chromosomes, and *one* provided evidence of non-disjunction of a sex chromosome. (4) One out of 52 second anaphases had 4 *n* chromatids.

The second metaphase with evidence of non-disjunction of a sex chromosome is the first of its kind found in a mammalian germ cell. The significance and origin of the apparently polyploid cells is discussed with the help of DNA replication studies on spermatogonia, but no definite conclusion was reached on the real nature of these cells.

Aneuploid zygotes in man have in a few cases been shown to be due to non-disjunction in the first or in both meiotic divisions in the male (review in FRØLAND, SANGER and RACE 1968). The quantitative significance of non-disjunction and non-reduction is difficult to assess, mainly because current techniques do not allow an adequate analysis of second meiotic metaphases. Presumptive polyploid cells have been observed in all stages of spermatogenesis in the mouse (FECHHEIMER 1961) and in man (SASAKI and MAKINO 1965; MCILREE et al. 1966) and triploid zygotes are not uncommon in man (CARR 1965).

During a study of the labelling patterns of the sex chromosomes in spermatogonial metaphases of the Chinese hamster we observed two apparently hexaploid spermatogonia, one second spermatocyte with a supernumerary chromosome, and several apparently polyploid cells. This led to a more systematic search for aneuploid and polyploid cells.

Material and methods

Seven adult male Chinese hamsters from an outbred strain were used. The animals were injected intraperitoneally two hours before sacrifice with 0.1 mg/kg body weight of Colcemid®. The mitotic karyotype was studied in cells from the bone marrow according to the method of TJIO and WHANG (1962). All the animals had a normal male karyotype, with 22 chromosomes. Air-dried meiotic preparations were made according to HULTÉN et al. (1966) in four of the animals. Squash preparations were made in the other three animals. The fertility of the animals was not known but all stages of spermatogenesis, including sperms, were observed in all preparations.

Results

The quantitative results are presented in Tables 1 and 2.

Spermatogonial metaphases were relatively few in the air-dried preparations. A total of 32 were

Table 1. Number of spermatogonial metaphases scored in air-dried (AD) and squash (S) preparations

Animal	Number of spermatogonial metaphases							
	Number of chromosomes 2 n				4 n	6 n	8 n	Total
	⩽20	21	22	⩾23				
1–AD	–	–	6	–	2	1	–	9
2–AD	–	1	6	–	2	–	–	9
3–AD	–	1	4	–	–	1	1	7
4–AD	–	1	6	–	–	–	–	7
Total	–	3	26	–	4	2	1	32
5–S	3	4	112	–	6	–	1	126
6–S	1	1	33	1	2	1	–	39
7–S	1	–	23	1	2	1	–	28
Total	5	5	168	2	10	2	1	193

Table 2. Number of analysed cells in first and second meiotic division

Diakinesis-First metaphase						Second metaphase							Second anaphase		
Number of bivalents					Total	Number of chromosomes						Total	Number of chromatids		Total
⩽9	10	11	⩾12	22		⩽9	10	11	12	⩾13	22		2 n	4 n	
–	3	50	–	–	53	1	3	88	1	–	–	93	17	–	17
2	3	81	–	3	89	5	3	195	–	–	2	205	15	–	15
–	1	10	–	–	11	1	1	40	–	–	2	44	6	1	7
1	–	20	–	–	21	–	–	49	–	–	1	50	13	–	13
3	7	161	–	3	174	7	7	372	1	–	5	392	51	1	52

analyzed, 22 of which were normal diploid (Fig. 1). Three had 21 chromosomes and the missing chromosome was different in each cell. Seven cells were apparently polyploid, four being tetraploid (Fig. 2 and 3), two hexaploid (Fig. 4 and 5) and one probably octaploid (Fig. 6). There were no endoreduplications.

In the squash preparations practically all the analyzable cells were spermatogonial metaphases; 193 of which were scored. Thirteen were apparently polyploid, 10 tetraploid, two hexaploid and one octaploid. Ten cells had a hypodiploid number, five with 21 chromosomes and five with less than 21. Two cells were found with a chromosome number between 23 and 44. In the squash preparations we did not find analyzable meiotic figures, but the slides had been selected for the study of the labelling pattern of the spermatogonial metaphases.

In the air-dried preparations 174 cells in *diakinesis—first metaphase* were analyzed. All of them had normal XY bivalents. We found three apparently polyploid first spermatocytes and these had normal bivalents and no multivalents (Fig. 7). A total of 10 cells showed a random loss of one or two bivalents. The morphological appearance and the aggregation of cells in leptotene—pachytene did not allow an evaluation of the ploidy in these earlier stages.

In *second spermatocytes* the X chromosome was in most instances clearly heteropyknotic in metaphase while both X and Y were apparently

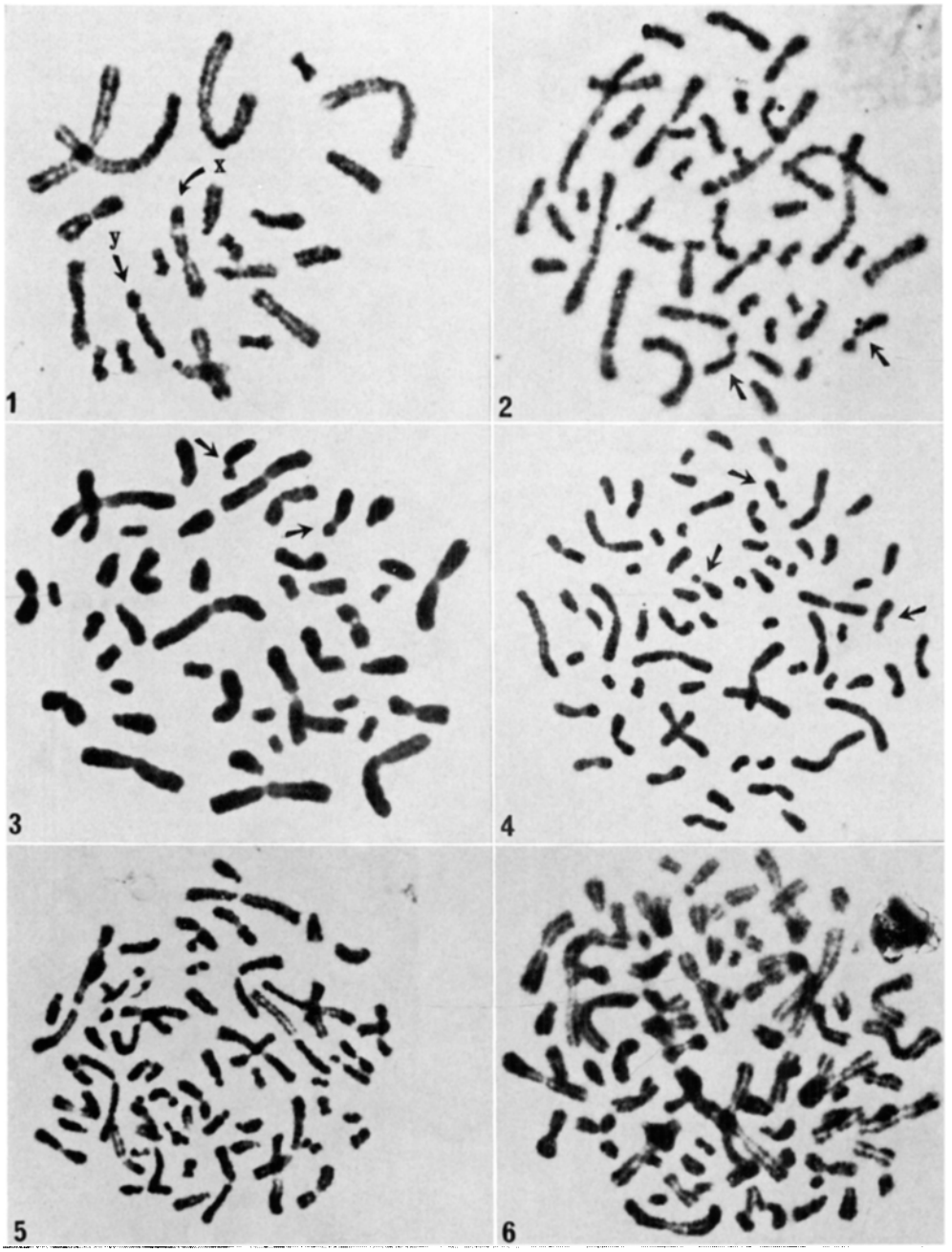

Fig. 1 – 6. – Fig. 1. Normal spermatogonial metaphase with 22 chromosomes. The X chromosome and the Y chromosome, with a secondary constriction on the long arm, are indicated by arrows. – Fig. 2 and 3. Spermatogonial metaphases with 44 chromosomes. The Y chromosomes are indicated by arrows. – Fig. 4 and 5. Spermatogonial metaphases with 66 chromosomes. In Fig. 4, the Y chromosomes are indicated by arrows. – Fig. 6. Octaploid spermatogonial metphase. The exact chromosome number could not be determined with certainty.

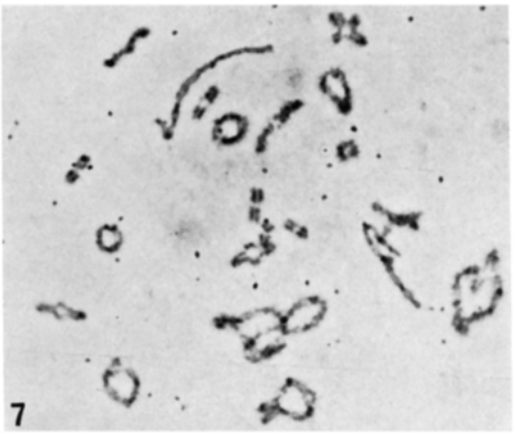

Fig. 7. First spermatocyte in diakinesis with 22 bivalents. The two lower bivalents were lying just outside the field and were photographed separately.

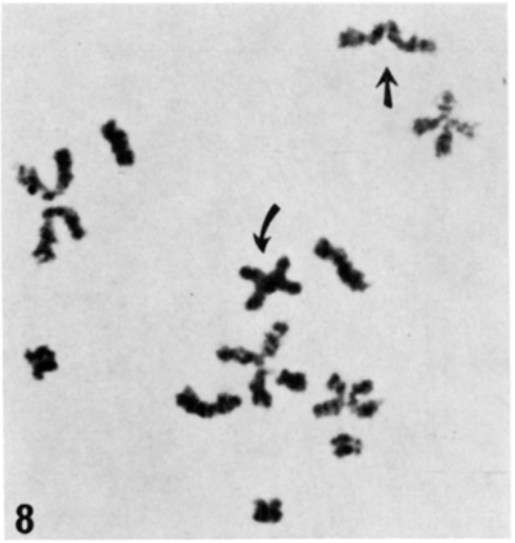

Fig. 8. Aneuploid second metaphase with 12 chromosomes including one X and one Y chromosome (arrows).

heteropyknotic in anaphase. We analyzed 392 second metaphases and 52 second anaphases. Five metaphases and one anaphase appeared polyploid (Fig. 9 and 10). Fourteen of the metaphases showed a random loss of one or more chromosomes. One had an extra chromosome, probably a sex chromosome (Fig. 8). Of the second metaphases 176 had an obvious X chromosome and 134 a Y chromosome while 82 cells could not be evaluated with certainty.

In an attempt to define the real nature of the polyploid cells we carefully screened the autoradiographic preparations of animals which had been injected in vivo with tritiated thymidine (see FRACCARO et al. 1969). None of the labelled tetraploid cells showed a differential pattern of replication of the two diploid complements (Fig. 11). On the other hand we repeatedly found obviously adjacent cells that were also synchronous in chromosome replication (Fig. 12).

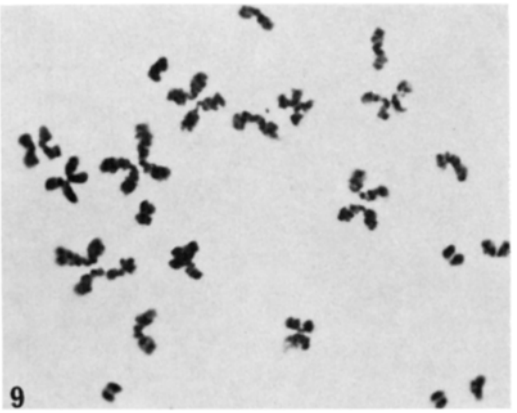

Fig. 9. Second metaphase with 22 chromosomes.

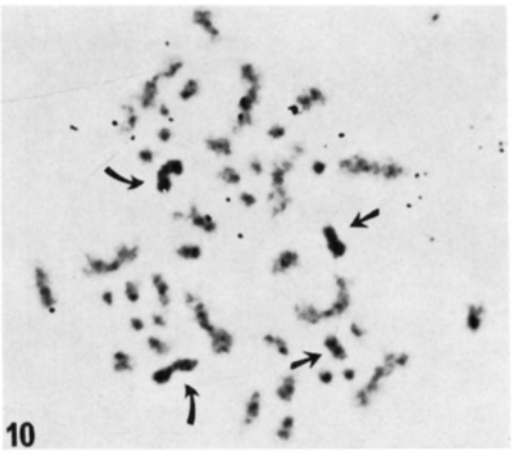

Fig. 10. Apparently diploid second anaphase. The heteropyknotic X and Y chromatids are indicated by arrows.

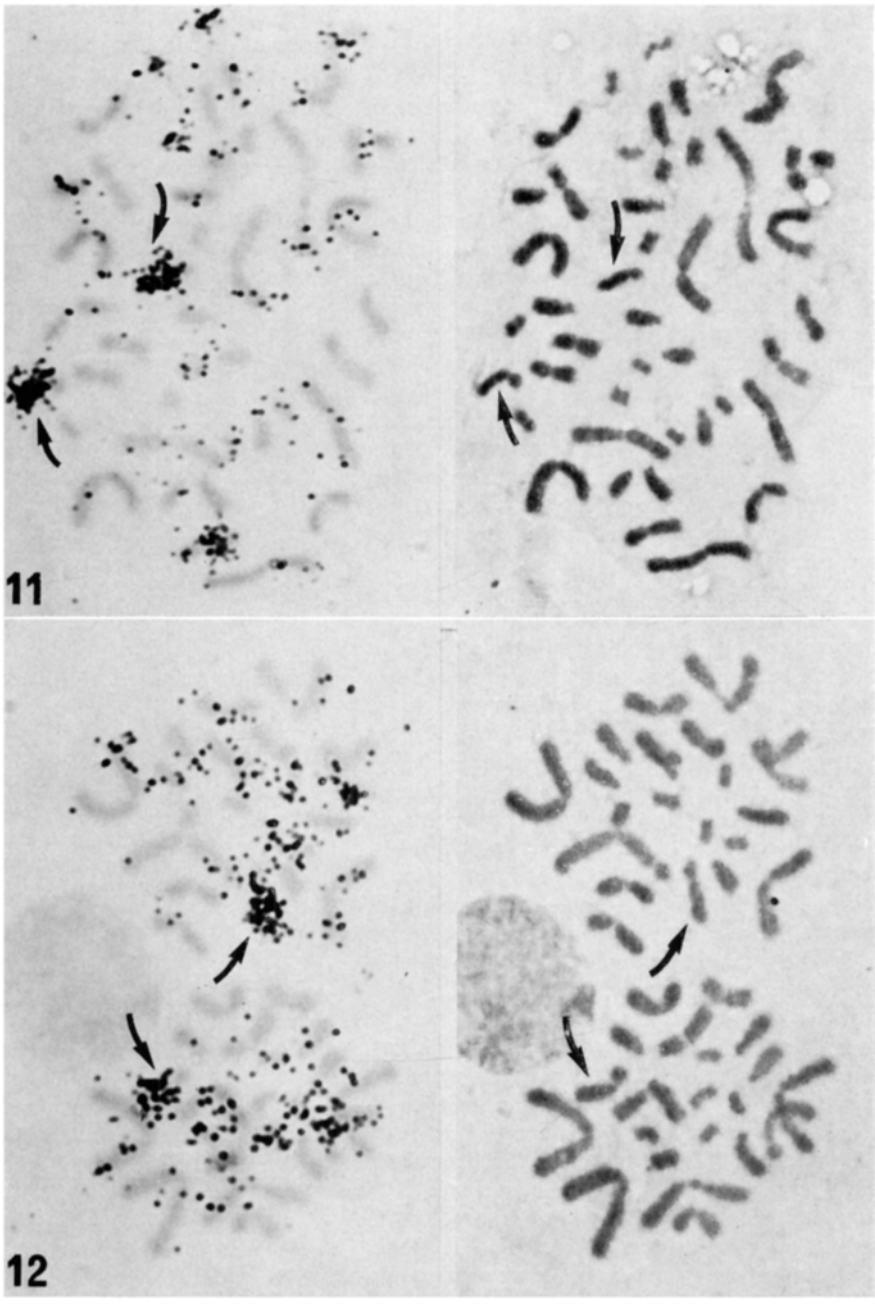

Fig. 11 – 12. – Fig. 11. Polyploid spermatogonial metaphase from an animal injected with tritiated thymidine for the last 9 hours. The two last replicating Y chromosomes (arrows) are synchronous. At the right side the same cell without grains. – Fig. 12. Two adjacent spermatogonial metaphases from the same animal of Fig. 11. Synchronous late replicating Y chromosomes indicated by arrows.

Discussion

As seen from Table 2 we found 15 aneuploid second metaphases out of 392. Loss of chromosomes is occasionally found using the air-drying technique and the observed 14 hypohaploid second metaphases can easily be explained on this basis. Gain of chromosomes is much more difficult to explain since they are very rare, indeed, in air-dried preparations where cells in division are very scattered on the slides. For this reason we find the one second metaphase with 12 chromosomes shown in Fig. 8 of interest and providing evidence of non-disjunction.

In the mouse OHNO, KAPLAN and KINOSITA (1959) failed to find evidence for errors of sex chromosomes pairing and/or segregation in 2192 meiotic figures. Similarly, ELIASSON et al. (1968) did not find aneuploid cells with extra chromosomes in studying meiosis of the dog.

The finding of apparently polyploid, especially hexaploid, spermatogonial metaphases and of polyploid first and second metaphases are difficult to interpret. One could simply dispose of these findings as artefacts. Since the cellular membranes are broken by the hypotonic treatment diploid cells lying close together might simulate a polyploid cell. However, the fact that the chromosomes in the apparently polyploid cells showed the same degree of contraction and synchrony in replication and seemed to be completely intermingled speak against the observation being an artefact. On the other hand, cells lying close to each other in the seminiferous tubules have highly synchronized cell cycles. If these cells stick together during the preparation of the meiotic slides, despite the attempts to obtain a suspension of single cells, polyploidy might be simulated. In this connection it should be noted that intracellular bridges between germinal cells have been demonstrated by FAWCETT et al. (1959) in testis of various species, and they are considered to be responsable for the synchronization among the cells of the germinal line (FAWCETT 1961). Thus, these bridges may be at least in part responsable for the occurrence of apparently polyploid cells.

In turn, the fact that no first spermatocyte had a multivalent speak against the existence of real polyploid spermatogonial metaphases unless we assume that these are eliminated. In conclusion, if polyploidy does occur, the origin of tetra- and octaploid cells can be explained on the basis of endomitosis, non-reduction or fusion of diploid cells. On the other hand, hexaploid cells could only originate either by fusion or unequal segregation of a highly polyploid cell in a multipolar division in analogy with the observations of PERA and SCHWARZACHER (1968). So far such mechanisms have not been described to occur in germ-line cells.

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