NON-SYNCHRONOUS SEGREGATION OF CHROMOSOMES IN THE MEIOSIS OF THE HOUSE MOUSE (*MUS MUSCULUS*)

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Introduction

During the studies on a new type of genetically determined variation in fertility in the house mouse (Rajasekarasetty, 1951, 1954, 1956 *a* and 1956 *b*) a detailed study of spermatogenesis was made in normally fertile males of the genotypes +/+, T/+, T/t^n and t^8/t^8. In general the spermatogenesis was similar to that studied previously (Federley 1919, Guthrez 1922, Masui 1923, Cox 1926, Painter 1927, Minuchi 1928, Cutright 1932, Crew and Koller 1932, Makino 1941 and Bryson 1944). Intensive search for cytological abnormalities in quasisterile and sterile males revealed that spermatogenesis was essentially similar to that of normals. The main deviation from normality was that the synchronous segregation of chromosomes to either poles was not universal as claimed by Makino (1941). There were seen both in controls and t^0t^3 males, both precocity and lagging of chromosomes during meiotic disjunction (Rajasekarasetty 1952 and 1953). Whether there is any difference in the incidence of such abnormality between the controls and quasisterile and sterile males and if so, could we attribute this anomaly to the interaction of t^0 and t^3 is the subject matter of this paper. Further the factors that are responsible for the reproductive impairment belong to a series of alleles at or near the " T " locus in the chromosome IX. (Dunn 1937, 1952 and 1956, Chesley and Dunn 1936, Dunn and Gluecksohn-Schoenheimer 1939 and 1950, Dunn and Gluecksohn-Waelsch 1950 and 1953). These alleles are normally maintained in balanced lethal lines in which both T/t^x and T/t^n are tailless, whereas, crosses between the two lines produce the compound t^xt^n distinguished by normal tails and impaired fertility. Since these balanced lethal systems feature

a prevention of recombination with in a definite region of the chromosome IX, it is suspected that this phenomenon is associated with an inversion in this chromosome. This project is undertaken to find out cytologically whether these compounds were inversion heterozygotes.

MATERIALS AND METHODS

The mice used were a part of the colony maintained by the department of Zoology, Columbia University, New York, U.S.A. 8 normal males, 24 quasisterile males and 9 completely sterile males were killed by percussion and the testes were fixed in Bouin's fluid (Allen's modificaion), San Felice, Champy's and Flemming without acetic acid. Squash preparations were made according to Slizinsky's method (Slizinsky 1949). Sections were stained in Feulgen, Iron Haematoxylin, Slizinsky's (1949) and counter stained in fast green.

OBSERVATION

During the detailed studies of meiosis in both fertile and reproductively impaired males special attention is paid to metaphases and anaphases to find the incidence of precession and lagging of chromosomes. It is observed that both long chromosomes (Figs. 1, 2, 5, 6, 7 and 16) and short chromosomes (Figs. 3, 4, 14, 15, 17 and 18) are amenable to precocious segregation to the poles while most of the chromosomes are still in the equator. Delay in reaching the poles resulting in laggards involved long autosomes (Figs. 11 and 12) and sex chromosomes (Figs. 9, 10 and 13). It is interesting to note that even small chromosomes (Figs. 21 and 22) show succession. Consequent on the delay of the long chromosomes in disjoining and reaching the poles, several anaphases give pseudobridge effects Figs. 10, 11, 12 and 13). It is also observed that precession and succession of chromosomes are met with in metaphase I and anaphase I only. No anaphase II and metaphase II showed non-synchronous segregation of chromosomes. Table I shows the counts and percentages of the precession and succession of chromosomes in the different stages of meiosis.

16.8% of first metaphases showed precession in controls while quasisteriles and steriles showed 18.4%. While 14.2% of first anaphases

showed lagging of chromosomes in controls, only 12.7% of anaphases showed lagging in the quasisterile and sterile males. Further the difference in percentage frequencies between the controls and $t^0 t^3$ and $t^1 t^3$ is found to be insignificant.

TABLE I

Table I showing the incidence and percentage frequencies of precocious segregation of chromosomes in metaphase I and II; and lagging of chromosomes in anaphase I and II.

Genotype	Fertility	Stage	Observed number	Number showing deviations	Percentage
+/+, T/+	Normal	Metaphase I	101	17	16.8
T/t^n, $t^8 t^8$		Metaphase II	50	00	0.0
		Anaphase I	105	15	14.2
		Anaphase II	67	00	0.0
$t^0 t^3$ &	Quasi-	Metaphase I	298	55	18.4
$t^1 t^3$	sterile &	Metaphase II	79	00	0.0
	sterile	Anaphase I	141	18	12.7
		Anaphase II	40	00	0.0

Of the 105 first anaphases examined, only one showed a true chromatid bridge with an acentric fragment. (Figs. 8 and 23). The incidence is too low to account for an inversion.

DISCUSSION

Since genic imbalance is one of the main agencies in bringing about sterility, chromosomal misbehaviour in disjoining during meiosis was suspected. An intensive search for chromosomal abnormalities in both the controls and reproductively impaired males of the genotypes t^0t^3 and t^1t^3 revealed that spermatogenesis was normal. The lack of synchronization of segregation of chromosomes to either pole is the main deviation from the work of Makino (1941) who claims that in the normal house mouse, the separation of chromosomes is synchronous, and " in going to poles, X and Y neither lag

behind nor precede the autosomes." ...."and any lagging of chromosomes is due to fixational artefacts." If this statement is accepted as universal, the deviations that were encountered by the author must be either due to the interaction of the two genes t^0 and t^3 or t^1 and t^3 or to the fixational defects. Studies on the incidence of precocity and lagging of chromosomes in both the controls and sterile and quasisterile males show that the difference in the percentage frequencies is not significant. This leads us to conclude that non-synchronous segregation is not associated with the above said factors. The second possibility is checked by paying special attention to fixing and staining the materials. This eliminated the fixational artefacts. Reference to literature showed that non-synchronous segregation has been reported in other mamals. (Painter 1924 in horse, Painter 1927 *a* in rodents, Allen 1918, Koller and Darlington 1934 in albino rat). This indicates that non-synchronous segregation is prevalent in mammals. Further Jaffe (1952) found non-synchronous segregation of chromosomes in the house mouse of the genotypes T/t^0, T/t^1 and T/t^8 and other controls. He reported that both anaphase I and anaphase II showed laggards. No such laggards were found by the author in the genotypes studied. It is also found that non-synchronous segregation has not led to meiotic disruption, since the daughter nuclei are formed after all the members of homologous pairs as well as X and Y reach the poles. (Fig. 19 and 20). While synchronous segregation is probably universal in the mice studied by Makino 1941 (wild forms of *Mus musculus M. mollossinus* and *M. Caroli* Bonhote), at least in the laboratory strains of *Mus musculus* of normal fertility and other genotypes maintained by Columbia University, New York, non-synchronous segregation is not unusual.

What makes the chromosomes lag or precociously segregate is not clear. Many interesting views have been held with regard to the behaviour of sex chromosomes in insects and other animals (Schrader 1928). Topographical relationships of chromosomes with reference to either poles could be the cause for precession, succession or synchronism (Piza 1945, referred to by Venkatasubba Rao 1958). Laggards that give pseudo-bridge effect could be due to stickiness of chromosomal ends of the separating homologues (Scultz and St.

Lawrence 1949). They could be due to precocity of extra chromosomal mechanism (Therman and Timonen 1951); or delay in terminalization of chiasmata and physical disturbances caused by the crowding of large number of bivalents in the equatorial plate (Darlington 1932) or delay in X and Y separation (Painter 1927). Differential response of the kinetochore of sex chromosome to the polar forces could be the cause of the differential behaviour of the sex chromosome (Venkatasubba Rao 1958). Since most of the views put forward are based on the behaviour of sex chromosomes, and the author finds that not only sex chromosomes but also long and short autosomes are involved in precession and succession, one is led to believe that the differential behaviour of chromosomes may be due to more than one cause.

In regard to the incidence of true chromatid bridges and acentric fragments, the percentage 0.95 is too low to account for a large inversion. Jaffe (1952) recorded a similar low percentage (about 1.4) in T/t^0, T/t^1 and T/t^8 males. Though the frequency of bridges is too low, the possibility of existence of a small inversion which allows lesser opportunity for intra inversional crossing over with subsequent formation of bridge and acentric fragment, cannot be ruled out. However Dunn (1956) recognizes the possibility that this "t" series must have occurred at different sites of mutation in the complex locus in the chromosome IX of the house mouse.

Summary

A detailed study of first metaphases, second metaphases, first anaphases and second anaphases in both the controls and sterile and quasisterile males reveals that non-synchronous segregation of sex chromosomes, as well as long and short autosomes is not uncommon in the strains of mice maintained by the Zoology Department, Columbia University, New York.

The difference in the percentage frequencies between the controls and t^0t^3 and t^1t^3 is found to be insignificant, indicating that precession and succession of chromosomes in the reproductively impaired males are not due to the interaction of the factors at the "T" locus.

The very low frequency of chromatid bridges and acentric fragments indicates that "t" series is not associated with a large inversion.

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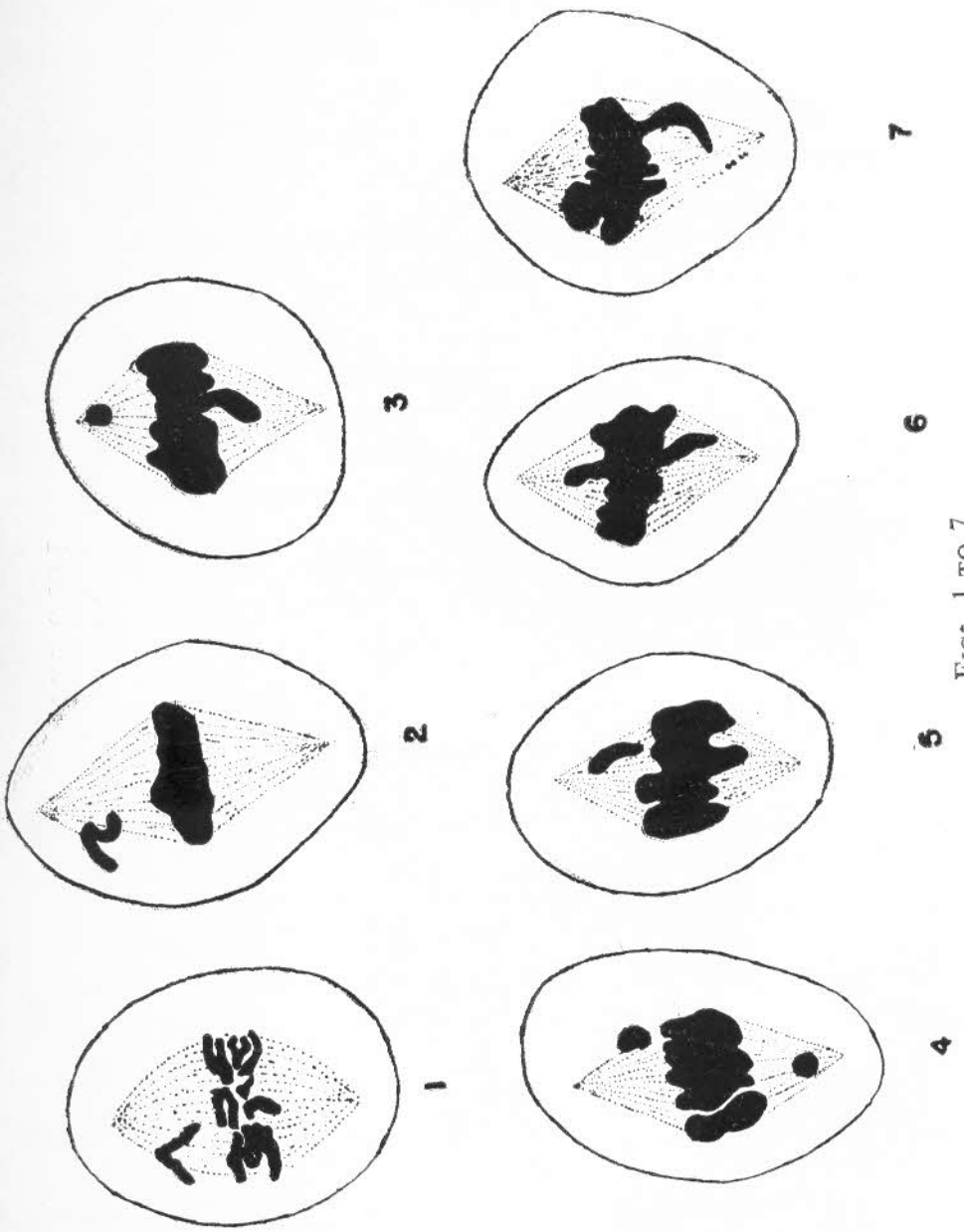
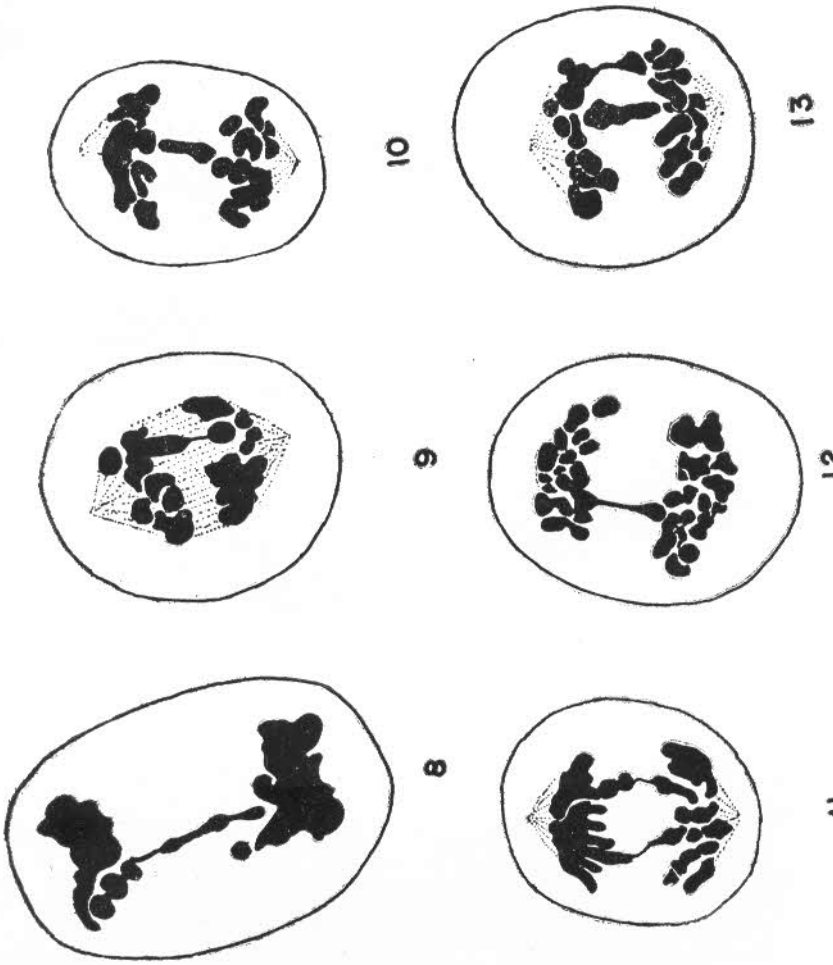
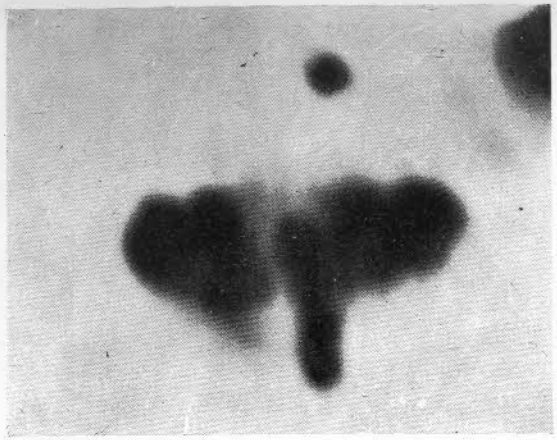


FIGS. 1 TO 7



Figs. 8 to 13



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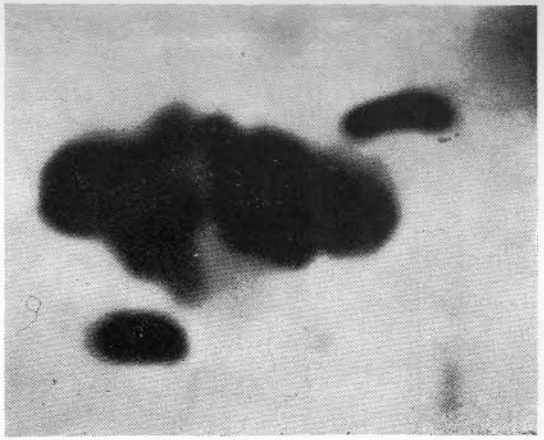

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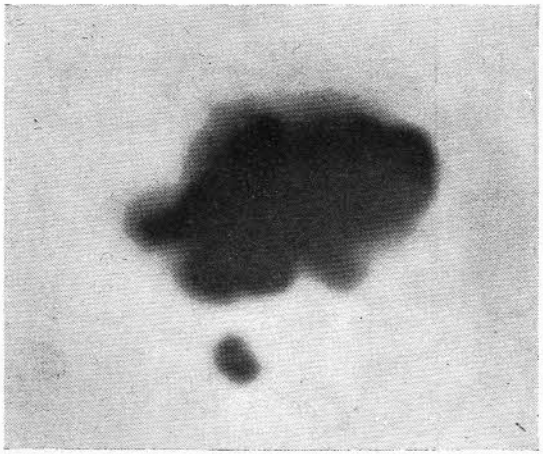

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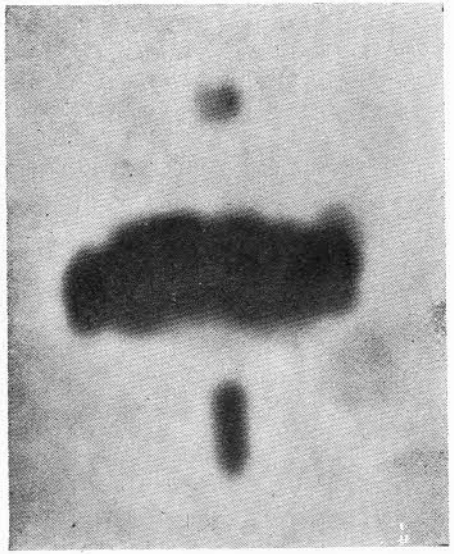

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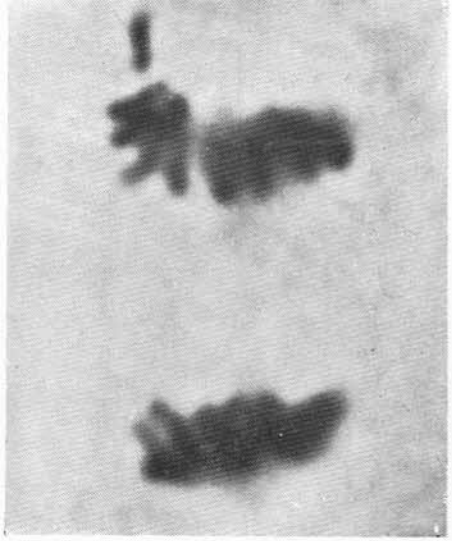

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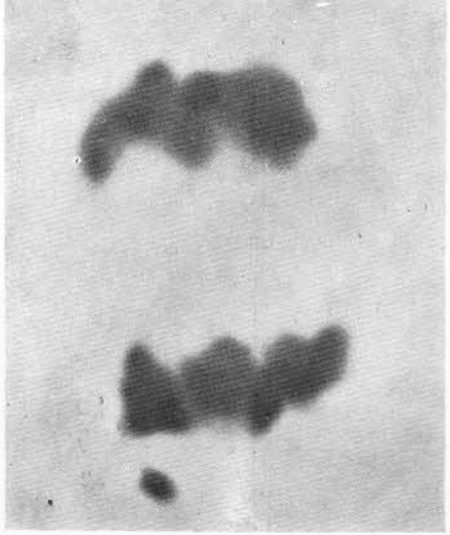

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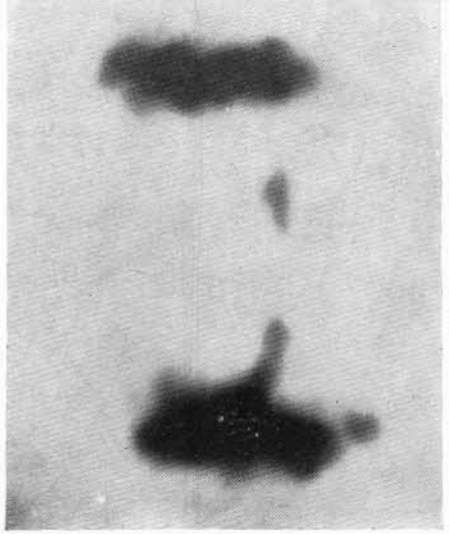

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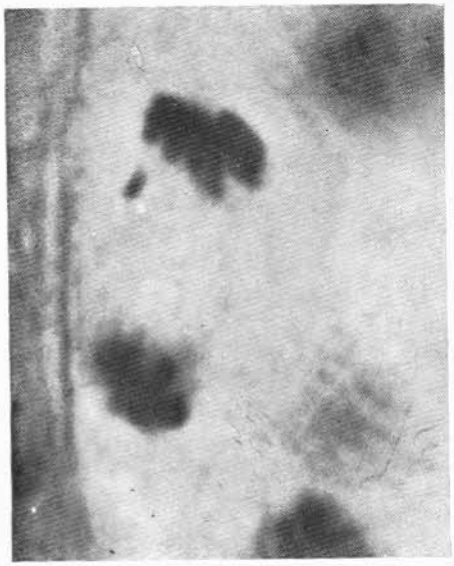

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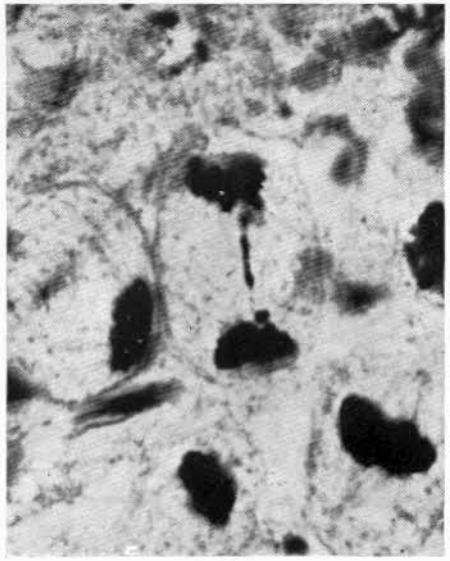

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Description of Figures

Figures 1 to 13. Were drawn with the help of Camera Lucida. × 1,650. Microphotographs 14 to 22 × 6,000. Microphotograph 23. × 3,000. All figures and Microphotographs from Heidenhein's haematoxylin and fast green preparations.

Figures 1, 2 and 5. Metaphase showing precocious segregation of long chromosomes.

Figure 3. Metaphase I. Y chromosome has reached the pole and the X chromosome has left the equatorial plate.

Figure 4. Metaphase I. Two short chromosomes preceding the rest of the chromosomes.

Figures 6 and 7. Metaphase I. Long chromosomes leaving the equatorial plate.

Figure 8. Anaphase I. Showing a true chromatid bridge and an acentric fragment. (*See* Fig. 23).

Figures 9, 10 and 13. Anaphase I. X and Y chromosomes lag while the rest of the chromosomes have reached the poles.

Figures 11 and 12. Delayed segregation of chromosomes giving the pseudo-bridge effect.

Figure 14. Metaphase I. Precocious segregation of X and Y chromosomes.

Figure 15. Metaphase I. A short and a long chromosome preceding the rest of the chromosome complement.

Figure 16. Metaphase I. Two autosomes leaving the equatorial plate.

Figure 17. Metaphase I. A short chromosome leaving the equatorial plate.

Figure 18. Metaphase I. One short autosome and the Y chromosome reaching one pole and the X chromosome reaching the opposite pole while the rest of the chromosomes are still in the equator.

Figures 19 and 20. Late Anaphase I. Showing a precociously segregated chromosome at one pole.

Figures 21 and 22. Anaphase I. Showing lagging of short chromosomes.

Figure 23. Anaphase I. Showing a true chromatid bridge with an acentric fragment.