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COLCHICINE-INDUCED ABNORMAL MEIOTIC CHROMOSOMAL SEGREGATION IN PRIMARY OOCYTES OF THE CHINESE HAMSTER

Part II. ANAPHASE LAGGING

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Summary A single dose of 3 μg/g b.w. colchicine was intraperitoneally injected to female Chinese hamsters with a normal estrous cycle at the onset of the formation of the first meiotic spindle. Morphologically abnormal secondary oocytes having one or two extremely large first polar bodies occurred frequently, *i.e.*, 47 (11.3%) out of 416 oocytes. In 30 of them, chromosome analysis was successful with both the oocytes and their giant polar body/bodies. In 25 cases, the abnormal chromosome segregation between the oocyte and its giant polar body/bodies could be ascertained. Three types of abnormal segregation were classified. One of them was of nondisjunction and the remaining two of anaphase lagging. The fate of the lagging chromosomes was observed. They were included neither in the oocytes nor in their polar body, and eventually were degenerated within perivitelline space. The behavior and the fate of the lagging chromosomes and the mechanism of the giant polar body formation were discussed.

INTRODUCTION

According to Hamerton (1971), the failure of inclusion of chromosomes in either daughter cell is defined as "anaphase lagging." As mentioned in Part I of our paper (Sugawara and Mikamo, 1980), the *in vivo* administration of colchicine to primary oocytes of the Chinese hamster induced frequently hypohaploids having a small number of dyads in the secondary oocytes. Their occurrence was apparrently related to the formation of a giant first polar body/bodies which were especially frequent in the treated oocytes. In many of them, the dyads lost from the oocytes were not included in their polar body. Therefore, the phenomenon was clearly different from the so-called nondisjunction, but very much like the anaphase lagging. This prompts us to present the observation on the process of loss of dyads

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and the fate of the lagging chromosomes which have never been reported in the first meiotic division of mammalian oocytes.

MATERIALS AND METHODS

The same materials and methods as those of Part I of the paper (Sugawara and Mikamo, 1980) were used. The collected secondary oocytes were treated with 0.5% trypsin to remove the cumulus cells, and their morphological features were examined under a dissecting microscope before their chromosome slides were made.

RESULTS

In the experimental group, morphologically abnormal eggs having one or two extremely large first polar bodies were found frequently (Fig. 1). Whilst, in the control group, such oocytes occurred very rarely (Table 1).

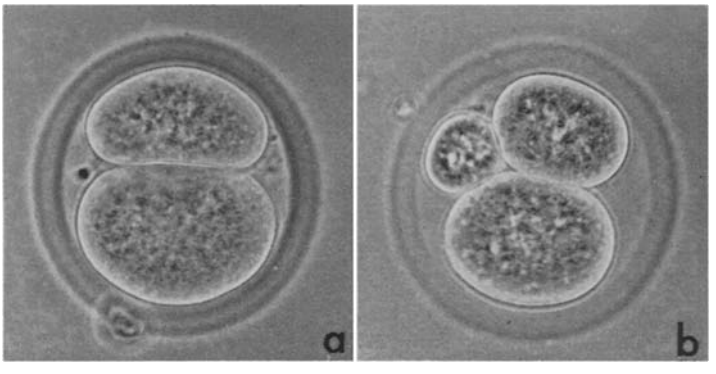

Fig. 1. A giant polar body (a) and bodies (b) caused by a single dose of 3 μg/g b.w. colchicine which was injected intraperitoneally to the Chinese hamsters with a normal estrous cycle at the onset of the formation of the first meiotic spindle. ×420.

Table 1. Incidence of morphologically abnormal oocytes II with an extremely large first polar body/bodies in the Chinese hamsters injected with 3 μg/g b.w. colchicine.

	No. of animals	No. of oocytes collected (mean)	No. of normal oocytes (%)	No. of abnormal oocytes (%)
Control group	254	1,912 (7.5)	1,899 (99.3)	13 (0.7)
Experimental group	55	416 (7.6)	369 (88.7)	47 (11.3)
χ^2-test				$p<0.001$

In the morphologically abnormal oocytes, hypohaploids were far more frequent than hyperhaploids in both groups, especially in the experimental group (Table 2). As shown in Fig. 2, hypohaploids with a very small number of dyads were often found. This strongly suggests that there must be a particular mechanism by which hypohaploids are formed more frequently than hyperhaploids, when the giant polar body is formed.

Karyotype analysis was usually impossible with the normal polar body, but often possible with the giant polar body. In 30 of those 40 cases shown in Table 2, the chromosomal complements of both the oocytes and their giant polar body/bodies were analyzed. Thus, the abnormal chromosomal segregation between the

Table 2. Incidence of aneuploids in morphologically abnormal oocytes II of the Chinese hamsters injected with 3 μg/g b.w. colchicine.

	No. of eggs karyotyped	Normal (%)	Aneuploidy		
			Hyper-haploidy (%)	Hypo-haploidy (%)	Total (%)
Control group	12	9 (75.0)	0	3 (25.0)	3 (25.0)
Experimental group	40	11 (27.5)	3 (7.5)	26 (65.0)	29 (72.5)
χ^2-test					$p<0.01$

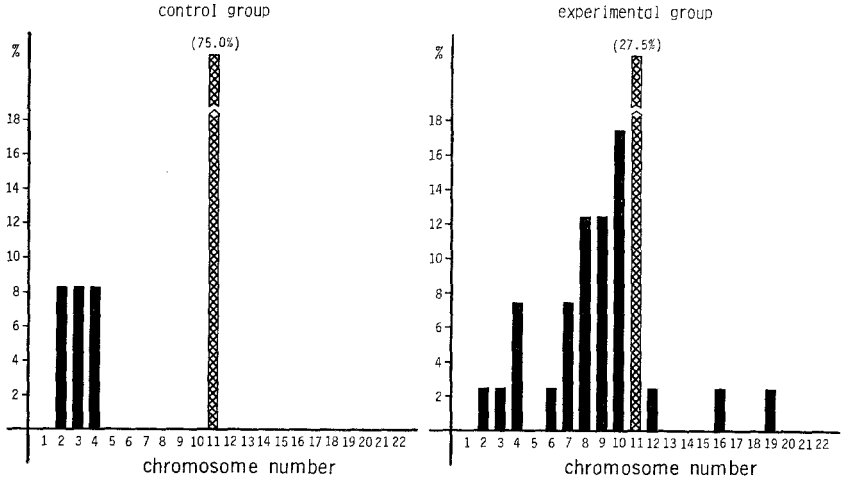

Fig. 2. The frequency distributions of aneuploids in the control and the experimental group are shown only for the morphologically abnormal oocytes having a giant polar body/bodies. Note the high incidence of hypohaploids in both groups.

oocytes and its giant polar body/bodies could be ascertained in twenty-five morphologically abnormal eggs. These aneuploid ova could be classified into three types as to their aspects of abnormal chromosome segregation and each was subdivided into two, those with one polar body and those with two polar bodies (Table

Table 3. Abnormal chromosome segregations in the oocytes II having one or two giant polar bodies in the Chinese hamsters injected with 3 μg/g b.w. colchicine.

Type	Case No.	Oocyte				P.B.I-1				P.B.I-2				Total (2n)				Eliminated, degenerating chromatin masses
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	
I-a	1	3	6	4	6	1	0	2	0					4	6	6	6	
	2	1	3	3	3	3	3	3	3					4	6	6	6	
I-b	3	2	5	5	4	2	0	0	1	0	1	1	1	4	6	6	6	
	4	2	3	3	2	1	1	2	2	1	2	1	2	4	6	6	6	
	5	1	3	2	2	1	2	3	2	2	1	1	2	4	6	6	6	
	6	1	3	2	2	1	1	1	3	2	2	3	1	4	6	6	6	
	7	2	2	2	2	1	2	2	2	1	2	2	2	4	6	6	6	
II-a	8	2	3	3	2	2	3	3	3					4	6	6	5	+
	9	1	3	3	3	1	2	2	1					2	5	5	4	+
	10	1	2	3	3	1	3	2	2					2	5	5	5	+
	11	2	3	2	2	1	3	1	2					3	6	3	4	+
	12	0	2	2	3	2	3	3	3					2	5	5	6	+
II-b	13	1	2	1	3	1	1	2	3	1	2	3	0	3	5	6	6	+
	14	1	2	1	2	1	0	2	3	2	3	1	0	4	5	4	5	+
III-a	15	2	2	3	3	2	2	1	2					4	4	4	5	
	16	1	3	3	3	2	3	1	0					3	6	4	3	
	17	2	2	3	2	1	2	2	3					3	4	5	5	
	18	1	2	3	3	2	3	1	1					3	5	4	4	
	19	1	3	2	2	1	2	2	3					2	5	4	5	
	20	1	2	2	3	2	2	2	0					3	4	4	3	
	21	1	3	2	1	1	2	3	1					2	5	5	2	
	22	1	2	1	0	2	3	3	3					3	5	4	3	
	23	2	1	0	1	2	3	3	3					4	4	3	4	
	24	1	1	0	0	2	3	3	4					3	4	3	4	
III-b	25	1	1	0	2	2	3	2	1	1	2	3	1	4	6	5	4	

Numbers and morphology of chromosomes of each group are as follows in the haploid condition: A, two large metacentric chromosomes; B, three medium metacentric chromosomes; C, three medium acrocentric chromosomes; D, three small metacentric chromosomes.

3). As mentioned earlier, the way in which chromosomes were lost from both the oocytes and their polar body will be shown in the following analysis.

Type I: The dyads lost in an oocyte were detected in its polar body, and *vice versa* (Type I-a). These were the cases of nondisjunction. In Type I-b, the full number of dyads was also present, but they were distributed randomly among the oocyte and two polar bodies. This phenomenon is obviously different from Type I-a in its process to form an aneuploid oocyte, but may also be classified as nondisjunction.

Type II: Some dyads were missing either in the oocyte or in the polar body (Type II-a) or bodies (Type II-b), but degenerating chromatin masses were found in the furrow between the oocyte and the polar body/bodies (Fig. 3). This phenomenon is the so-called anaphase lagging in which aberrant chromosomes are included in neither daughter cell.

Type III: Some dyads were also missing, but the degenerating chromatin masses were not found (Fig. 4). This type may be the case of an advanced stage of Type II. But, it is very likely that the degenerating chromatin masses in the perivitelline space were lost accidentally, since the zona pellucida had been dissolved during the process of chromosome preparation. Yet, this may also be the case of anaphase lagging.

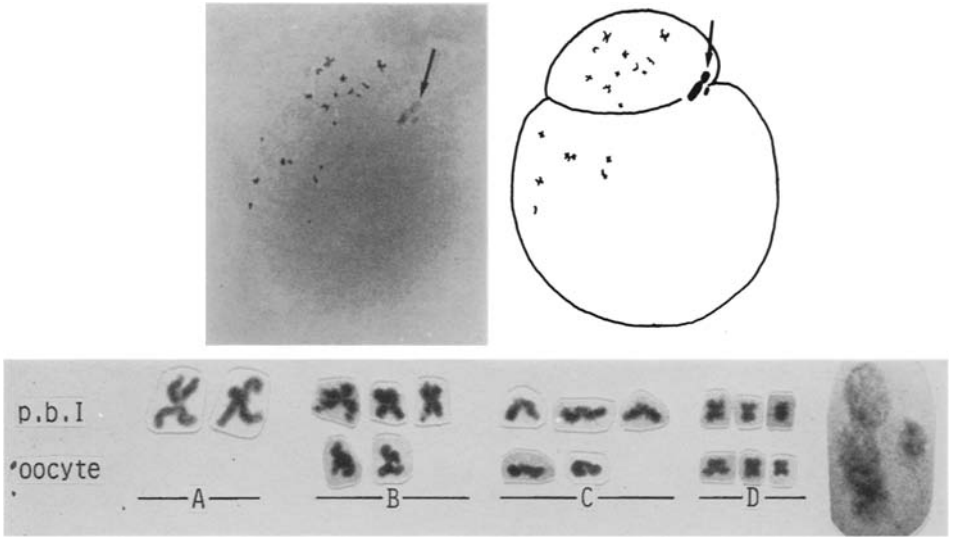

Fig. 3. Metaphase II of an egg of Type II-a (Case No. 12 in Table 3). Left: Photographed after Giemsa stain. Right: A diagram of the same picture emphasizing characteristic features for the chromosomal segregation and cytoplasmic condition of both the oocyte and its giant polar body. Note the degenerating chromatin masses (arrow) staying in the furrow between the oocyte and the giant polar body, and showing the fate of anaphase-lagging chromosomes. Bottom: Karyotypes of the oocyte and of the giant 1st polar body. The degenerating chromatin masses may include two large metacentric chromosomes (A-group), a medium metacentric one (B-group), a medium acrocentric one (C-group) which are all missing from the oocyte.

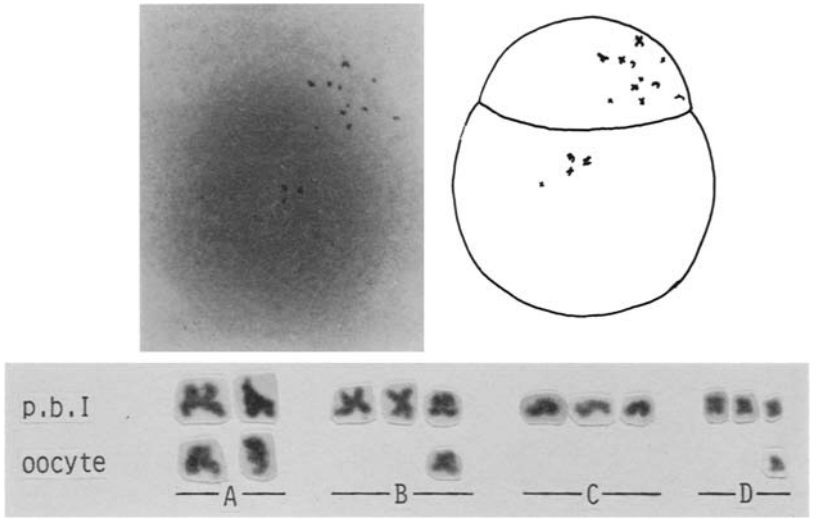

Fig. 4. Metaphase II of an egg of Type III-a (Case No. 23 in Table 3). The illustrations are of the same nature as those in Fig. 3. Note that seven dyads are missing in the oocyte, but no degenerating chromatin mass is seen. This suggests that the lagging chromosomes which had been eliminated into perivitelline space were lost after the zona pellucida was removed during the course of chromosome preparation.

DISCUSSION

It was shown that the maximum genome mutagenicity of colchicine at our selected dose caused the elimination of a large number of chromosomes from the egg body, in association with the formation of a giant polar body/bodies (Tables 2 and 3, Fig. 2). The chromosomal elimination into the perivitelline space appeared to take place following the anaphase lagging. According to Hamerton (1971), the failure of inclusion of chromosomes in either daughter cell is "anaphase lagging." However, as to the fate of lagging chromosomes, Kato and Sandberg (1968) observed that in human somatic cells *in vitro*, some of them might be included in the daughter cells to form micronuclei during the interphase, and to transform into pulverized chromosomes at the following metaphase. The lagging chromosomes have been seen frequently in colcemid-treated somatic cells *in vitro* (Kato and Yoshida, 1970).

The fragmented small pronuclei of female nuclear origin could be induced in fertilized eggs by the colchicine or colcemid which had been injected to female mice and rats at about the time of ovulation (Austin and Braden, 1954; Edwards, 1958, 1961; Piko and Bomsel-Helmreich, 1960; McGaughey and Chang, 1969). According to Edwards (1958), the micropronuclei were formed with the chromosomes (dyads) which had been scattered within ooplasm following the destruction of the second maturation spindle by colchicine. He observed that some of these scattered

chromosomes which were located near the egg surface could be extruded into the perivitelline space together with a small amount of ooplasm, possibly owing to the continued movements of the egg cytoplasm before fertilization. In our experiment, however, such a complete destruction of the spindle did not take place, possibly because of the limited dosage of colchicine. Yet, the micropronucleus was seen occasionally in eggs inseminated after the same treatment (Sugawara and Mikamo, unpublished data). This suggests that some of the lagging chromosomes left near the equator of the spindle at telophase might be included in the egg body, while the majority of them were eliminated into the perivitelline space when the giant polar body/bodies were formed, and eventually degenerated forming the chromatin masses.

One of the most striking effects of colchicine on the primary oocytes was the frequent formation of the giant polar body/bodies (Table 1). A large first polar body has been seen among the eggs maturing *in vitro* in the mouse (Donahue, 1970) and in the golden hamster (Plachot *et al.*, 1978). A large second polar body was observed in ovulated mouse eggs having been subjected to heat treatment (Braden and Austin, 1954). These were all thought to be owing to the migration of the maturation spindle toward the center of the eggs. In addition to this, however, Plachot *et al.* (1978) proposed the importance of the orientation of the spindle axis when the polar body is cut off.

The giant polar body formation in our experiment may have resulted either from the displacement of the spindle toward the center of the egg or from the incorrect orientation of the spindle axis. However, the effect of colchicine on cytoplasmic microtubules is also conceivable as the cause, because the microtubules have been said to be associated with the microfilaments which form the contractile ring in cell division (Van Den Brenk and Stone, 1974). Cremer *et al.* (1976) induced cell fragmentation in human fibroblasts *in vitro* by the use of colchicine, suggesting that it disordered cytokinesis by forming several contractile rings, possibly through its effect on cytoplasmic microtubules. Formation of a tripolar spindle may be one of the possible mechanisms causing plural giant polar bodies, for it has been induced *in vitro* by colcemid in the Chinese hamster's somatic cells (Stubblefield *et al.*, 1967).

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