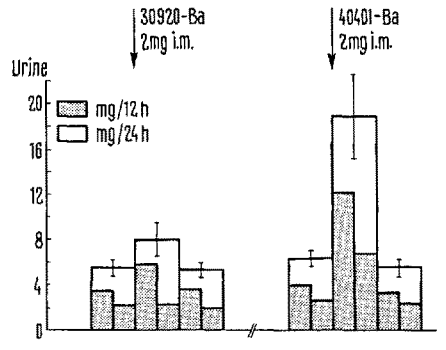

Fig. 1. Urinary excretion of 17-hydroxycorticoids (HOC) following injection of 2 mg 30920-Ba and 40401-Ba. Dark columns 17-HOC mg/12 h, empty columns 17-HOC mg/24 h.

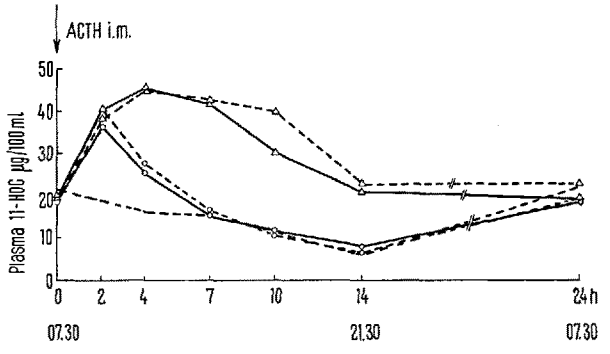

Fig. 2. Mean values of plasma 11-hydroxycorticoids (HOC) following injection of 1 mg or 2 mg 30920-Ba and 40401-Ba with regard to normal diurnal variation. o——o 1 mg 30920-Ba, o----o 2 mg 30920-Ba, △——△ 1 mg 40401-Ba, △----△ 2 mg 40401-Ba, ---- spontaneous diurnal variation.

Plasma-11-hydroxycorticoids after i.m. injection of synthetic ACTH derivatives at 07.30.

	1 mg 30920-Ba	2 mg 30920-Ba	1 mg 40401-Ba	2 mg 40401-Ba
0 h	18.8 ± 3.3	18.8 ± 1.3	20.2 ± 2.6	19.4 ± 2.3
2 h	36.5 ± 1.5	40.3 ± 9.0	40.5 ± 4.1	38.3 ± 5.3
4 h	25.3 ± 1.4	27.8 ± 4.5	45.6 ± 1.7	45.0 ± 5.2
7 h	15.5 ± 0	16.6 ± 2.3	42.0 ± 2.5	43.1 ± 9.4
10 h	11.8 ± 0.25	10.8 ± 1.9	30.1 ± 5.9	39.9 ± 14.8
14 h	8.0 ± 0.16	6.5 ± 1.1	20.9 ± 6.2	22.8 ± 20.0
24 h	18.8 ± 3.5	19.3 ± 1.8	19.6 ± 1.3	23.2 ± 3.8

Mean values and standard deviation in µg/100 ml.

after stimulation is 2.4 mg for 30920-Ba and 12.5 mg for 40401-Ba, indicating that the increase produced by the synthetic derivative is 5 times higher. With regard to spontaneous diurnal variation, measured in 12 h urine specimens, the rise of the excretion of 17-hydroxycorticoids can only be observed in the first 12 h period after 30920-Ba, whereas after 40401-Ba it lasts for 24 h.

The determination of plasma 11-hydroxycorticoids gives more information on the dynamic of adrenal stimulation. In the Table the mean values and standard deviation of the mean of the 2 groups at dose levels of 1 mg and 2 mg of both derivatives are plotted. The group consisted of 5 and 8 persons respectively. It can be seen that the increase after both preparations is the same during the first 2 h. The effect of the synthetic derivative, however, lasts for more than 14 h after injection. The only difference between the 2 dose levels of 40401-Ba is that after 2 mg the standard deviation of the mean is greater, due to individual differences. It has to be noted that already 7 h after the injection of 30920-Ba the values of plasma 11-hydroxycorticoids follow the normal diurnal rhythm of the adrenals.

The curve of all mean values of the plasma 11-hydroxycorticoids is shown in Figure 2. The difference between the 2 derivatives is the same, independent of the 2 dose levels. The calculation of the area by planimetry using normal diurnal variation as baseline, gives for 1 mg 40401-Ba an area which is 4.8 times larger and for 2 mg 40401-Ba an area which is 4.5 times larger than the corresponding values of 30920-Ba.

In conclusion we may state that replacement of L-serine[1] by D-serine[1] and arginine 17,18 by ornithine 17,18 enhances the adrenocorticotropic effect not only in animals but also in humans. The synthetic derivative is about 5 times more active. The effect is probably due to a different enzymatic degradation.

Zusammenfassung. Am Menschen wurde die adrenocorticotrope Wirkung von β^{1-24} Corticotrophin Tetracosactid und D-Serin[1]-17,18 Diornithin β^{1-24} Corticotrophin nach i.m. Injektion untersucht. Das synthetische Derivat erwies sich als etwa fünfmal stärker wirksam als das natürliche Tetracosactid, hauptsächlich durch eine Verlängerung der Wirkungsdauer.

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Mechanism of Non-Disjunction of Meiotic Chromosomes and of Degeneration of Maturation Spindles in Eggs Affected by Intrafollicular Overripeness[1]

Non-disjunction of meiotic chromosomes is thought to be the cause of various types of trisomy and monosomy. Principal etiological factors for the non-disjunction during ovogenesis which have been postulated are (1) post-ovulatory overripeness of normally ovulated eggs[2,3], (2) preovulatory overripeness of normally matured primary ovocytes[3-6], (3) perhaps chronological ageing of ovocytes during the growth period which may occur in aged mothers.

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That pathological modifications in eggs caused by post-ovulatory (intrauterine or intratubal) overripeness and by preovulatory (intrafollicular) overripeness may be very similar is shown by comparison of the subsequent results occurring in cytoplasmic and nuclear elements of affected eggs and in the issued zygotes[7]. In the eggs of *Xenopus* which had been affected by intrafollicular overripeness, abnormal chromosomal behaviour during metaphases of the first and the second maturation divisions were observed and interpreted as of non-disjunction of chromosomes in the earlier studies[4,5]. Such behaviour was characterized by abnormal location of undivided tetrads and dyads in the spindles and their abnormally slanting position to the spindle axis.

The present report provides further evidence of the effect of intrafollicular overripeness on the polar spindles.

Materials and methods. Three female *Xenopus*, more than 8 years of age, had been kept over 1 year without ovulation. Injected with 500 IU of human chorionic gonadotropin each furnished over 1000 eggs, some normal, others dead or degenerating. As is well known, degeneration is caused by intrafollicular overripeness[4,5,7]. Ovulated eggs were collected from body cavity, upper part of oviduct and uterus 10 h after the injection, and immediately placed into Zenker's solution together with the excised ovaries. They were sectioned at 15 μ and stained with hematoxylin and eosin.

Results. Meiotic spindles of 338 externally normal looking eggs were observed, but only 179 could be evaluated regarding chromosomal behaviour. Spindles in side view were selected for a study of the structure, location and polarity of meiotic chromosomes. As summarized in Table I, in 3 of 8 cases of first meiotic metaphase spindles and in 20 of 171 cases of second meiotic metaphase spindles abnormal chromosomal behaviour was observed. This included the following:

In the first meiotic metaphase (Table II). (1) One tetrad is located close to the inner pole, far below the equatorial plane where all others are assembled (Figure 1). The displaced tetrad stands at an angle of about 45° to the main axis while all others are parallel to the spindle. (2) One tetrad is located near the outer pole and some are not polarized with slanting position to the spindle axis. (3) Three tetrads in the equatorial plane show lost polarization (Figure 2).

In the second meiotic metaphase (Table II). (1) One case with 2 dyads in opposite poles of a single spindle (Figure 3), and not polarized normally. (2) One case with 2 dyads nearly in the outer pole. (3) One case with 2 dyads half way between the outer pole and the equatorial plane (Figure 4). (4) Six cases with 1 dyad located near the outer pole, clearly separated from the other dyads at various distances (Figure 5). Most of the dislocated dyads show lost polarization. (5) Nine cases with 1 dyad near the inner pole clearly separated from the other dyads at various distances (Figure 6). Most of the dislocated dyads exhibit lost polarization. (6) Two cases with some dislocated and degenerating chromosomes. One of them shows a large sized spindle body (Figure 7).

The most frequent and characteristic abnormalities in these cases were precocious moving of undivided tetrads or dyads toward spindle poles and lack of polarization of abnormally located chromosomes.

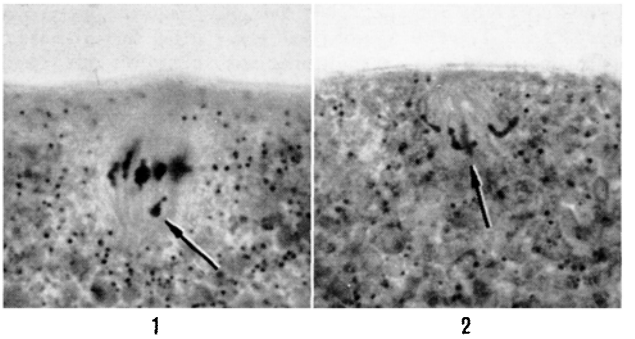
Table I. Frequency of cases with abnormal meiotic chromosomal behaviour in eggs collected from body cavities, upper part of oviducts and uteri

Sites of collection of eggs	No. of eggs studied	No. of eggs showing side view of spindle	No. of cases in first metaphase		No. of cases in second metaphase	
			normal	abnormal	normal	abnormal
Body cavity	130	83	5	3	66	9
Upper oviduct	101	41	0	0	36	5
Uterus	107	55	0	0	49	6
Total	338	179	5	3	151	20

Table II. Classified cases of the abnormal meiotic chromosomal behaviour in the eggs shown in Table I, and frequencies of precocious moving of undivided meiotic chromosomes to the outer or the inner poles of the spindle

Abnormal chromosome behaviour	No. of cases in first metaphase	No. of cases in second metaphase	No. of undivided chromosomes precociously moving to	
			outer pole	inner pole
Precocious moving of undivided chromosomes to the outer pole	1	8[a]	11	
Precocious moving of undivided chromosomes to the inner pole	1	9		10
Precocious moving of undivided chromosomes to 2 different poles		1	1	1
Losing polarization of chromosomes but not showing precocious moving	1			
Degeneration of chromosomes		2		
Total	3	20	12	11

[a] Including 2 cases with 2 dyads moving to the outer pole.



1 2

Fig. 1. A first meiotic spindle at metaphase in a body-cavity egg. Note the abnormal position of an undivided tetrad (arrow) and its lost polarization. × 1000.

Fig. 2. A first meiotic spindle at metaphase in a body-cavity egg. Note the lost polarization of 3 tetrads. The arrow points at a tetrad (slightly out of focus) which is normally polarized. × 1000.

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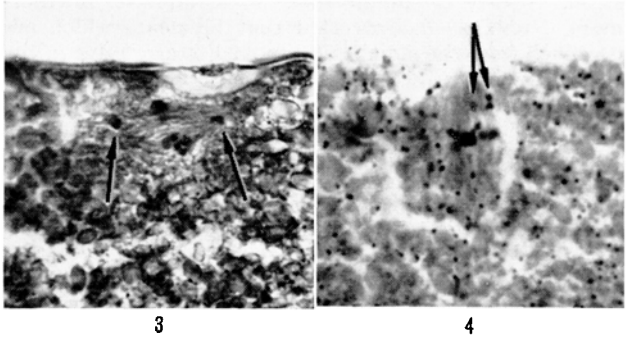
Other types of abnormality, i.e. degeneration of chromosomes and hypertrophy of spindles, appeared in 23 among 40 uterine eggs which showed various degrees of cytoplasmic degeneration (Figures 8A, 9A). Degeneration of chromosomes was characterized by deformation, fragmentation, and dissolution (Figures 8B, 9B). Hypertrophy of the spindle was observed in a majority of degenerating eggs (Figures 8B, 9B, compare with the spindles in Figures 1–6 at the same magnification). Disorganization of spindle fibres was one of the most interesting features. As is seen in Figure 9B, the polar ends of spindles often are scattered and not bundled. Collection of the fibres in central bodies is completely lost.

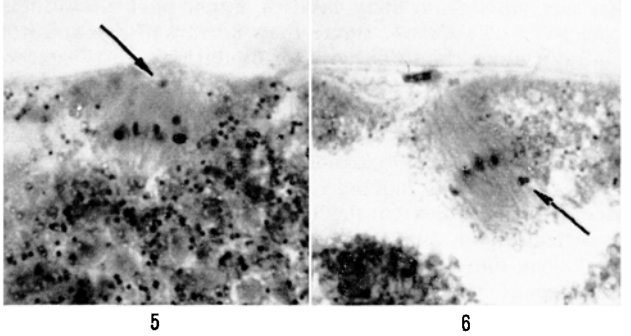
Discussion. Intrafollicular overripeness exerts widespread effects on ovular components, i.e. cytoplasm, chromosomes, spindle fibres, and central bodies. In cases of severe effect, ovocytes are completely degenerated and finally absorbed in the ovary. However, still relatively advanced cases can be ovulated and some reach the metaphase of the second maturation division. These eggs may



3 4

Fig. 3. A second meiotic spindle in a body-cavity egg. Note 2 dyads (arrows) in different spindle poles. The first polar body is shown near the spindle. × 1000.

Fig. 4. A second meiotic spindle at metaphase in an egg from upper oviduct. Two dyads (arrows) are about half way between the equatorial plane and the outer pole. × 1000.



5 6

Fig. 5. A second meiotic spindle at metaphase in a body-cavity egg. One dyad (arrow) is near the outer pole. × 1000.

Fig. 6. A second meiotic spindle at metaphase in a body-cavity egg. One dyad (arrow) is about half-way between the equatorial plane and the inner pole. Note the relatively clear structure of the dyad and its lost polarization. The first polar body is seen near the outer pole of the spindle. × 1000.

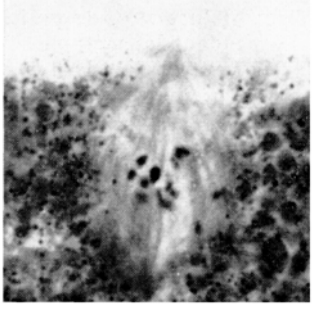
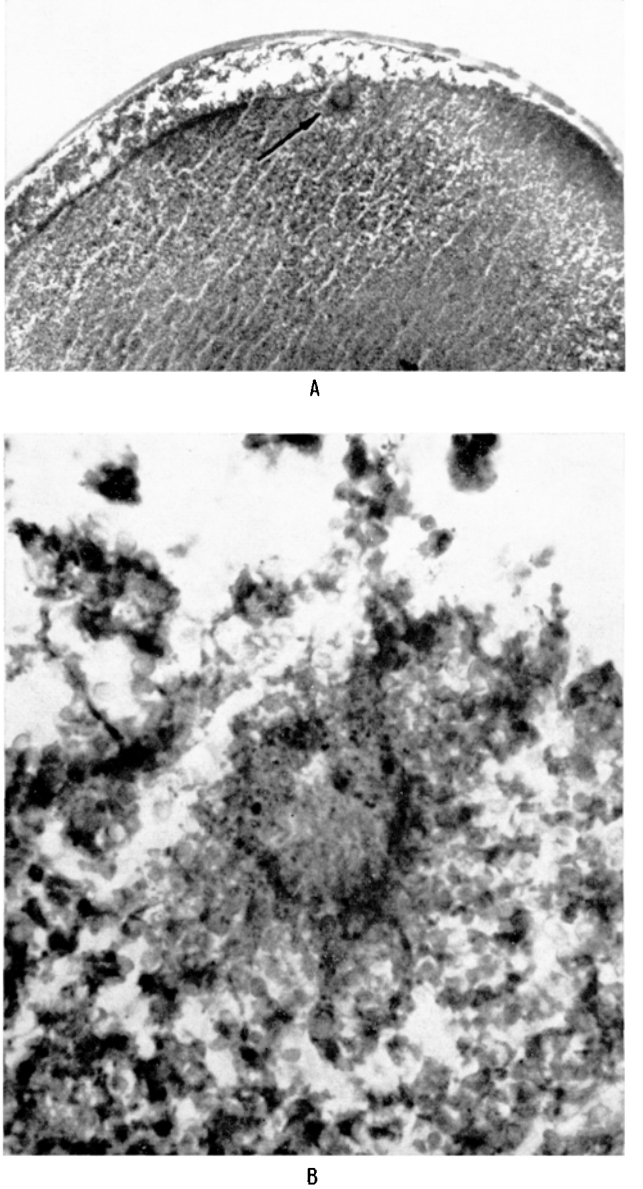


Fig. 7. A second maturation spindle in an uterine egg. Many chromosome dyads are located abnormally in the spindle. Note deformation and fuzzy appearance of the chromosomes and the large size of the spindle. × 1000.



A B

Fig. 8. (A) Section of a degenerating uterine egg through a second meiotic spindle near the equatorial plane. Note the degenerative change of cytoplasm near the egg surface. × 100. (B) The same spindle showing degenerated chromosomes and fragmented chromatin substance scattered in the spindle. Note the large diameter of the spindle (the same magnification as that of Figures 1–7). × 1000.

not be fertilizable, nor capable of development very far if fertilized. Relatively moderately affected eggs are fertilizable and develop into various developmental malformations[7] and chromosome anomalies[3].

The abnormal chromosomal behaviours, displacement and lack of polarization, are of special interest in the present study because they offer a possible explanation to the mechanism of non-disjunction of meiotic chromosomes. If spindle fibres connecting both poles are of normal quality, obviously the tetrads and dyads should be polarized and stay in the equatorial plane as all others do. Apparently, the connection with one pole has been lost or weakened. The cause of the non-disjunction of chromosomes seems related to degeneration of the spindle fibres. A firm attachment of chromosomes at the original characteristic position in the spindle was convincingly shown by NICKLAS and STAEHLY[8] in a recent experimental study of interference by micromanipulation with the normal process of division of grasshopper spermatocytes. The fact that prolonged or severe manipulation during metaphase could produce only a small increase in the kinetochore-to-pole distance, in other words, stretching spindle fibres was not easily performed, indicates a strong rigidity of spindle fibres in metaphase. Furthermore, NICKLAS[9] has revealed that bivalents which are detached from the spindle and moved to any place in the cell with a micro-needle at metaphase always return to their characteristic metaphase position. Such experimental evidence may support the interpretation that the poleward displacement and lack of polarization of the tetrads and dyads in the material are pathological and are an embodiment of the processes occurring in the non-disjunction of meiotic chromosomes.

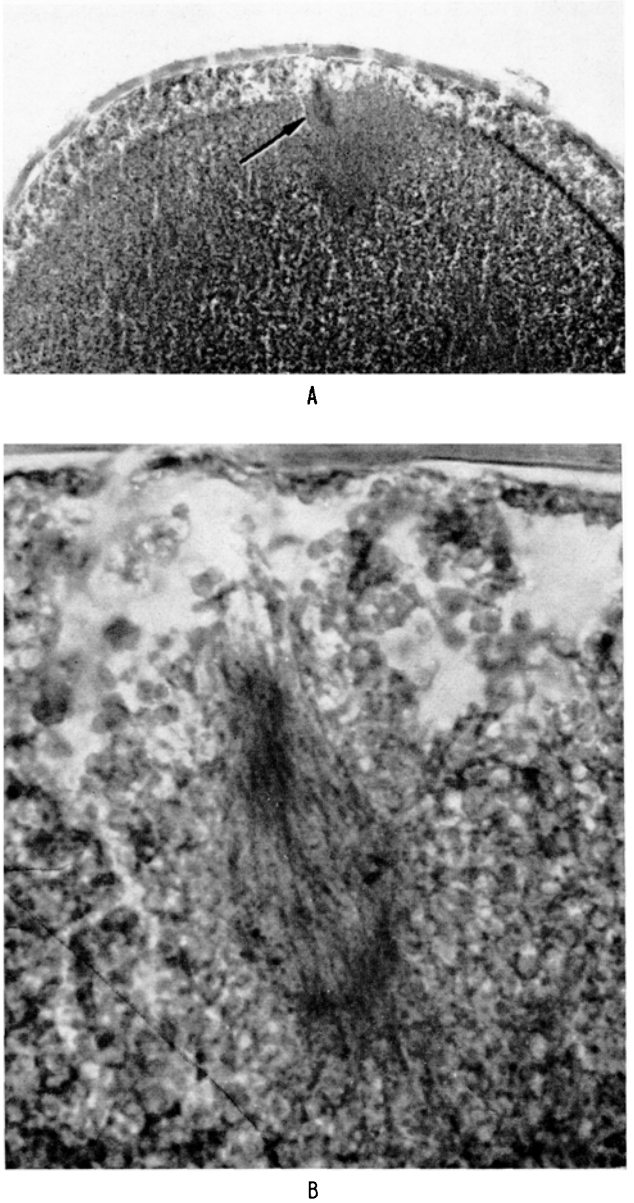


Fig. 9. (A) Section of a degenerating uterine egg through the second meiotic spindle. Cytoplasm is degenerated near the egg surface of the animal hemisphere. × 100. (B) The same spindle showing disorganization of spindle fibres at poles and discontinuity of the fibres with central bodies. Degeneration of chromosomes and enlargement of size of the spindle are seen also in this case. × 1000.

As the result of overripeness, plasma viscocity in the egg decreases[10]. In many among the degenerating uterine eggs, the cytoplasmic degeneration was especially advanced at the surface of the animal hemisphere (Figures 8A, 9A). Possibly, the hypertrophy of the spindle (Figures 8B, 9B) was caused by liquefaction which occurred foremost at the animal pole of the affected eggs. WITSCHI and LAGUENS[3] have suggested that such a factor may contribute to the deterioration of polar and cleavage spindle fibres.

Summary. Effects of intrafollicular overripeness of primary ovocytes on meiotic spindles were studied in the eggs collected from body cavities, upper part of oviducts and uteri of 3 *Xenopi* (more than 8 years of age and not induced more than one year for ovulation). In 179 externally normal eggs which were sectioned to show side view of the spindle, 3 cases (out of 8) in the first meiotic metaphase and 20 (out of 171) in the second metaphase exhibited abnormal chromosomal behaviour. This included precocious moving of 1 or 2 undivided tetrad or diad chromosomes to the outer or inner pole, lack of polarization of chromosomes, and degenerative changes of chromosomes. In obviously degenerating uterine eggs, the degenerative changes of spindles were found to be deformation, fragmentation, and dissolution of chromosomes, disintegration of spindle fibres especially at polar ends, and hypertrophy of spindles. From the observation, it was concluded that the cause of the non-disjunction of chromosomes may be related to degeneration of the spindle fibres.

Résumé. La dégénérescence des fibres en fuseau est proposée comme un facteur responsable de la non-disjonction des chromosomes méiotiques. Cette hypothèse est basée sur l'observation, dans les œufs affectés par l'hypermaturité intrafolliculaire, de positions chromosomiques anormales pendant les métaphases méiotiques et de changements dégénératifs dans les fuseaux méiotiques.

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