On induced fragmentation of ovarian oocytes

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Summary. Fragmentation of oocytes was induced in the ovary, via ovulation suppression, by administration of dehydroepiandrosterone acetate (DHA-Ac) to mature cycling rats. The maximal fragmentation ratio, 15.0 ± 3.2%, was obtained by 10 mg/100 g b.wt/day for 7-day administration of DHA-Ac. The relationship of fragmentation to the first stage of meiosis is also discussed.

Fragmentation of oocytes in the ovary[2] as well as in the Fallopian tubes is a well known phenomenon, although the cause and precise morphology require further clarification. Fugo and Butcher[3,4] examined fragmented oocytes in rat Fallopian tubes after ovulation suppression by sodium pentobarbital injections. They suggested that the increase in the number of fragmented oocytes in the tubes was attributable to the delayed ovulation, and fragmentation was a resultant form of overripened oocytes in the ovary. Their work was very suggestive. If anovulation should confer a capability for future fragmentation on ovarian oocytes, is there any reason that only ovulated ova should express the capability? If fragmentation of oocytes should really be induced through the indirect condition, anovulation, any chemical or physical agents which are able to induce anovulatory cycles should cause fragmentation, depending on the duration of anovulation.

In previous work[5] the author noticed an increase in the number of fragmented oocytes in the ovary under administration of dehydroepiandrosterone acetate (DHA-Ac). The present research was planned to examine the working hypotheses mentioned above, using DHA-Ac as the ovulation suppression agent.

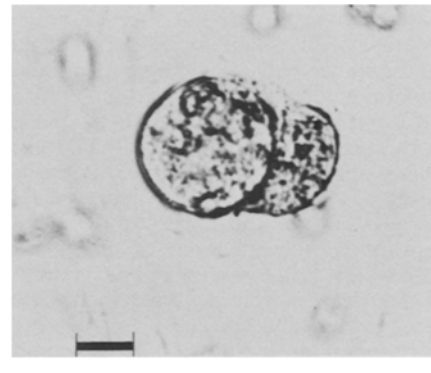
Materials and methods. 22 rats of the Wistar strain, aged from 8 to 12 weeks, were assigned for the present study. They were kept in a room with the temperature at 24 °C, and the lights were switched on at 07.00 h and off at 19.00 h. Solid laboratory chow and water were supplied ad libitum. Vaginal smears were taken every day before and during the experiment. Only animals with 4- or 5-day sexual periodicity were used for the experiment.

DHA-Ac was administered s.c. 1 mg/100 g b.wt/day and 10 mg/100 g b.wt/day for 4 and 7 consecutive days respectively. On days 5 and 8 some animals were killed. In the 10-mg group most of the animals showed constant diestrus smears until day 5, while the smears in the 1-mg group were less constant. Control animals received injections of the vehicle only and were sacrificed on appropriate days, when the diestrus smears were obtained.

The animals were killed using ether. After extirpation of the ovaries the surrounding tissues were removed onto a filter paper. The ovary was placed in a plastic dish (3.5 cm in diameter) filled with 5.0 ml of physiological saline solution (0.9% NaCl) and was randomly punctured 200 times by a multi(7)-barreled needle. The solution containing liberated oocytes was transferred to a test tube and 0.5 ml of 1% aceto-orcein was gently added. The dish was washed thoroughly with 2.5 ml of physiological saline solution and this solution added to the previous solution in the test tube. The tube was then centrifuged at 400 rpm for 5 min. The supernatant was discarded and 0.5 ml of the sediment was gently dropped and spread on a glass slide with a cover slip. The slit between the glass slide and the cover slip was adjusted to 300–400 μm and fixed by paraffin wax before the experiment. A normal microscope and a Nomarski differential interference contrast illumination with an Olympus photomicroscope (VANOX) were used for oocyte observation.

Fragmentation is a very conspicuous phenomenon and is easily differentiated from other morphological features of oocytes. However, when a fragmented oocyte appeared as the 2-cell stage, the daughter cell could not morphologically be discriminated from the 1st polar body. An uneven cleavage of an oocyte producing a daughter cell with 20–30 μm in diameter was regarded as a progressing stage of the 1st meiotic division and was excluded from the counting of fragmentation on a practical basis.

Results. The table presents the ratio of the appearance of fragmented oocytes in the 3 experimental groups. The ratios in the control and 1 mg × 4-d group did not show any statistical difference ($p > 0.05$), but comparisons between the control and the other administration groups revealed various differences ($p < 0.01$). Among the 4 administration groups the mean appearance ratios showed tendencies to increase according to days of administration and the doses



A fragmented oocyte with a diameter of 25 μm. The bar signifies 10 μm.

Appearance ratio of fragmented oocytes

	Control N = 6	1 mg/100 g b.wt/day × 4 days N = 4	1 mg/100 g b.wt/day × 7 days N = 4	10 mg/100 g b.wt/day × 4 days N = 4	10 mg/100 g b.wt/day × 7 days N = 4
Total oocytes observed	1008	576	702	859	736
Fragmented oocytes	25	46	80	100	110
	(2.5 ± 2.4)	(8.0 ± 5.3)	(11.4 ± 1.4)	(11.6 ± 4.5)	(15.0 ± 3.2)

N, Number of animals. ( ), Ratios of fragmentation expressed as the mean ± SD percent of the recovery.

per day, but statistics revealed no significant difference in comparisons of any 2 administration groups ($p > 0.05$).
The figure presents the configuration of a fragmented oocyte with 25 µm diameter.
Discussions. Administration of DHA-Ac enchanced fragmentation ratios of ovarian oocytes. Considering that sodium pentobarbital administration also has the same effect, the capability for oocyte fragmentation was postulated to be given not directly by the agents, but be given indirectly through the anovulation induced by them.
As far as the dose and the period of exposure are sufficient for the induction of definite anovulatory cycles, the ratio will rise. But the rise is not proportional to the period of anovulation, or has a limit, as was revealed by the insignificant differences in the ratio in the treated groups. The limitation seems to be a reflection of a limitation of the number of ovarian oocytes which are destined to undergo fragmentation. It is quite natural to consider that only oocytes toward ovulation will acquire the capability for fragmentation, because fragmentation is a resultant phenomenon of repeated cell divisions after resumption of cell division, whether mitotic or meiotic. However, our laboratory has proof that even oocytes with diameters of 25 µm or so in the ovary of rats with regular sexual cycles have a possibility of fragmentation (fig.). Therefore, if the hypothesis that the fragmented oocytes have been in a process of resumed meiosis before the fragmentation was accepted, it should also be accepted that such small oocytes had resumed meiosis and had been going to participate in ovulation until the fragmentation occurred. The present study cannot provide sufficient information to answer the question, and it is a problem for future work to determine whether fragmentation occurs only in oocytes which have been in meiotic progression for ovulation, or whether it occurs in other immature resting stage oocytes as well by an unknown mechanism.

1 Acknowledgments. The author would like to thank Professor T. Matsuda in the Department of Anatomy I for his valuable advices and Proff. E. Nishida and K. Akasofu in the Department of Obstetrics and Gynecology, Kanazawa University, for instructive suggestions and for kindly supplying DHA-Ac.
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PRO EXPERIMENTIS

A method for injection and transplantation of nuclei and cells in *Drosophila* eggs

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Summary. Microinjection of *Drosophila* eggs using the principle of thermal expansion is described. The eggs, mounted on cover slips with adhesive, are slightly dried to avoid the formation of exudate and then covered with fluorocarbon oil. Injections are made with a specially designed electrically controlled micropipette.

Injection into *Drosophila* eggs has been considered to be difficult because of the small size of the eggs, the internal pressure and a rather resistant vitelline membrane. Actually, size should not deter an adept micromanipulator. As for internal pressure, it can be overcome by a slight drying of the egg. A sharpened micropipette easily pierces the membrane. In her pioneering work on nuclear transplantation in *Drosophila* eggs, Geyer-Duszynska[1] pointed out the difficulties of the operation and proposed the use of viscous fluorocarbon oil to alleviate some of the damage due to injection. Since then, other authors have reported injecting eggs[2], but they have never described their techniques in enough detail to be easily followed.
The most common procedure of microinjection, using a small syringe attached to the micropipette with a capillary, presents certain difficulties for injecting eggs. The movement of liquids in the micropipette cannot be controlled easily and it is difficult to measure accurately the amount of material injected or aspirated. I devised, therefore, a microinjection system using the principle of thermal expansion[3,4]. The micropipette is sealed at its wider end to provide a small reservoir which is filled with oil. A resistance wire loop, heated electrically, warms the reservoir so that the expanding oil forces the liquid out of the pipette (fig. 1). Heating is controlled with a variable transformer. When the current is reduced, the reservoir cools and the contracting oil lets liquid reenter the needle. Since the contents of the pipette are not compressible, back pressure cannot force material into the needle, and injection can be made against external pressure. The micropipette is mounted on the micromanipulator (de Fonbrune) so that it reaches the preparation at an angle of 15°. The technique has been described in detail, including instructions for fabricating the micropipette[4].
Positioning of eggs for injection. Maintaining the eggs in place for microinjection has been done by some experimenters with a microsuction cup. This requires another micromanipulator assembly and makes manipulation rather tedious. I found that the eggs could be stuck to a streak of adhesive spread on a cover slip and remain attached under fluorocarbon oil (Voltalef oil, Ugine Kuhlmann; Société PROLABO, 12, rue Pelée, F-75011, Paris, France). This makes it possible to immobilize a row of eggs and to inject one after another in 1 session. The adhesive is applied as a solution, prepared by dissolving the glue of a suitable adhesive tape in heptane (e.g. Barnadher electric tape, Rubafix toile adhésive); use about 4 cm of the tape for 1 ml of solution. A piece of 0.2 mm copper wire placed on the cover slip helps to spread the glue solution and to retain later the oil cover over the eggs (fig. 2). The cover slip is then stuck to a microscope slide with a droplet of oil. Dechorionated eggs are placed on the sticky surface along one side of the copper wire in proper orientation in groups of 5 or 6.
Drying of eggs. The slide with the eggs is placed in a desiccator containing calcium chloride or silica gel. The