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.

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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

container is kept at 25 °C to ensure reproducible drying. Depending on the external temperature and humidity, the drying time may vary from 5 to 10 min. If the eggs are not dry enough, turgor will force the ooplasm out during pricking. Overdrying produces softish eggs, causing high mortality. Not all eggs dry at the same rate: the vitelline membrane prevents evaporation less in younger eggs.
The slide is then covered with Voltalef 10S oil (or a mixture of 10S and 20S for higher viscosity), so that it spreads about 2 mm beyond the eggs. It is important that the oil layer is not too thick since the reservoir of the micropipette should not be submerged (fig. 2).

Injection. Droplets of the liquid to be injected are deposited in spaces between groups of eggs (fig. 3a) under oil. The surface of the cover slip should be previously siliconized to prevent spreading of the droplet. The preparation is inserted into the plastic chamber affixed to the stage of a compound microscope[4] and moved with the stage movement to bring the row of eggs into the optical field of a 4× objective, while the pipette is brought near to them with coarse micromanipulator controls. Further manipulation is made by using 10× and 40× (long frontal distance) objectives and the fine controls of the micromanipulator.
The micropipette should have a diameter of 5–10 µm, delivering 100–200 pl (1–2% of egg volume) at full load. Stepwise increase in electric current permits the injection of fractions of this volume into each egg. The injection liquid is aspirated from the droplet and the egg brought by the stage movement in line with the pipette. The pipette is made to penetrate the vitelline membrane and enter the egg with a short stab using the forward movement of the micromanipulator. With transformer controls, the desired quantity of the liquid is injected and then the pipette removed with a slow motion. The exact volume injected can be determined by measuring a droplet of liquid expelled into oil by the same voltage difference. The next egg is then brought into the field and the operation repeated. 5–6 eggs can be injected with 1-pipette-full of liquid. After this, the pipette is refilled from the next droplet. Used pipettes can be cleaned by aspirating alternately hypochlorite and hydrogen peroxide (3%).

Post-operative care. The oil cover gives the injection wound sufficient protection. If the wound comes in contact with an aqueous liquid, cytoplasm oozes out. Cytoplasm can also be expelled if the oil level is lowered, compressing the egg by surface tension. The oil also helps to protect the egg from desiccation and from rehydration in a humid atmosphere. It is relatively permeable to oxygen, but if the oil layer is too thick, the egg may suffer from anoxia. In this case abnormal embryos are formed. In order to ensure proper humidity and oxygenation, the slide with eggs should be put into a container lined with wet filter paper and filled with about 50% oxygen. The container has to be kept at constant temperature to prevent condensation of vapor on the preparation due to temperature fluctuations. When the eggs are ready to hatch, the coverslip is removed from the slide and put on nutrient medium in a small Petri dish. Some larvae may stay entrapped in the oil and have to be transferred to the nutrient medium.

Nuclear transplantation in fertilized eggs. Recipient eggs should be in early division stages. Since it is time-consuming to select young eggs at the proper stages, all freshly laid eggs can be used and undesirable eggs removed or destroyed after injection. After dechorionated eggs are dried, donor eggs are placed without drying between the groups of recipient eggs in the proper orientation (fig. 3b). Alternatively the recipient eggs can be placed on a sticky band along the edge of the cover slip, while the donor eggs are on the edge of another cover slip facing it (fig. 3c).

Nuclei are transplanted with a pipette having a diameter of 10–15 µm. With a wider pipette, there is less risk of damaging the nuclei, but egg mortality increases rapidly with diameters of 20 µm and more. The best source of nuclei is the syncytial blastoderm where the resting nuclei can be clearly seen at the edge of the egg while mitotic stages appear blurred.
The heated pipette is introduced into the donor egg in such a way that the opening is turned towards the superficial nuclear layer of the blastoderm (fig. 4). Nuclei and cytoplasm are sucked into the pipette by reducing the current. Nuclei can be readily seen in the capillary of the needle as

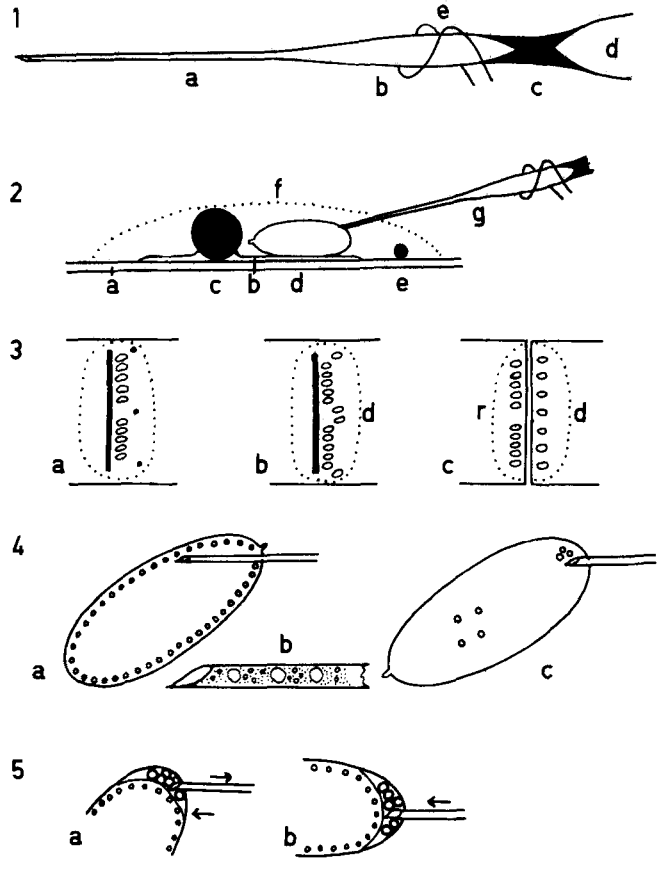

Figure 1. Micropipette. Micropipette is pulled from a 1.2–1.4 mm capillary (d) to make a reservoir (b) of diameter 100–200 µm, about 2 mm long, occluded by melting at one end (c) and tapering into injection needle (a). A heating wire loop (nichrome wire, diameter 0.05 mm, 550 ohms/m) surrounds the reservoir (e). The pipette is bevelled by grinding with a diamond wheel and a sharp point made with microforge.

Figure 2. *Drosophila* egg in position for injection; a) cover slip, b) sticky surface, c) copper wire, d) egg, e) liquid droplet, f) oil cover, g) pipette.

Figure 3. Slides with eggs positioned for microinjection. Copper wire and liquid droplets are in black. Oil cover is indicated with stippling; a) slide with droplets b) slide with donor eggs (d), c) alternate arrangement of recipient (r) and donor eggs (d).

Figure 4. Transplantation of nuclei into posterior end of the egg. Eggs are positioned at an angle of 60° to the copper wire; a) needle enters the donor egg, b) nuclei inside pipette, c) nuclei are injected into recipient egg.

Figure 5. Transplantation of pole cells; a) the donor is positioned at 45°, the egg is pierced at lower arrow and the pipette worked towards pole cells, b) the recipient is in line with the pipette.

transparent spheres (do not confuse with yolk granules which are smaller and more opaque). Some 5–10 nuclei are taken up and then injected into recipient eggs in twos or threes. After some practice, it may be possible to inject only 1 nucleus. The same donor egg can serve as a source of nuclei for several injections if one picks nuclei each time from an area which was not injured. After injections are done, the donor eggs should be removed from the slide to avoid confusion.

Transplantation of pole cells. Recipient eggs should be at syncytial blastoderm stages (stages 11–13[5]). Slight drying will retract the ooplasm and leave a free space near the pole cells. The eggs are oriented parallel to the direction of the pipette.

The best source of donor pole cells is the late blastoderm stage. The egg should be oriented at a wide angle to the pipette. One pierces the egg on the side and works the needle to the base of the pole cells by pushing and pulling (fig. 5). The cells are aspirated, the pipette withdrawn and inserted into the posterior pole of the recipient egg. Since the needle usually penetrates deeply into the egg after piercing the vitelline membrane, it has to be retracted to bring the opening just under the membrane. If the egg has been properly dried, it will accept all the cells injected. Post-operative care is as for other injections.

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Improving laboratory safety: replacing benzene by toluene in phosphate analyses

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The determination of organic phosphorus compounds using phosphate analysis reported by several authors involves the use of an organic solvent mixture containing benzene. This old but very accurate and sensitive method is widely used for several enzymatic assays, including 5′-nucleotidase (EC 3.1.3.5)[1,2] and 2′,3′-cyclic nucleotide 3′-phosphodiesterase (EC 3.1.4.37)[3]. Concerned with the danger of acute and chronic benzene toxicity, we tried substituting toluene for benzene. We obtained identical phosphate values using either benzene or toluene in the solvent mixture. Therefore, we recommend discarding benzene in phosphate analyses and replacing it with toluene, which is much less toxic.

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A field-portable racetrack and timer for measuring acceleration and speed of small cursorial animals[1]

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Summary. We describe a relatively inexpensive, field-portable racetrack and timer that can be used to measure acceleration and maximum speed of small cursorial animals. Procedures for interfacing the racetrack to microprocessors are also outlined.

Measurements of acceleration and velocity are basic to various physiological, morphological and ecological studies. Average velocity can be estimated easily by connecting 2 photocell detectors to an electronic clock; but acceleration and maximum velocity require more elaborate equipment. The usual technique involves frame-by-frame analysis of films or videotapes. However, this method is relatively expensive, tedious, and inconvenient for field research. More importantly, data reduction is not immediate.

Here we describe a field-portable racetrack and timer that instantaneously provide data necessary to quantify acceleration and velocity. The timer and photocells cost between US$250 and $500 (exclusive of labor), depending on options selected. We also outline procedures and programs for attaching the racetrack to microprocessors to speed data recording and analysis. Complete circuit diagrams (timer and photocell stations) and computer programs are available on request from the senior author.

Apparatus. The racetrack consists of a narrow runway that contains several photocell stations positioned at known distances along the walls of the track. An animal is placed just behind the 1st photocell beam and is stimulated to sprint. When the animal breaks the 1st beam, a multichannel timer is activated; and the time when each subsequent beam along the track is broken is stored into memo-