IN VITRO INCORPORATION OF URIDINE-H^3 INTO DEVELOPING FRUIT FLY OOCYTES

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The developing egg of *Drosophila melanogaster* consists of a 16-cell nest surrounded by an envelope of follicle cells. The 16 cells are daughters which arise by four consecutive divisions of an oogonium. Fifteen of the daughter germ cells differentiate into nurse cells and nourish the most posterior daughter germ cell which becomes the oocyte. The nurse cell chromosomes elongate by uncoiling and concurrently undergo a series of 8 or 9 endomitotic doublings which produce polytene chromosomes whose multiple strands become more and more loosely associated as the nucleus grows. The nurse cell nucleus contains large amounts of ribonucleoproteins not confined to a single structure, but rather localized in very dense, ribbon-like chains of blobs. This compound plasmosomal element presumably originates by the fusion of numerous plasmosomes arising from multiple chromosomal plasmosome organizers. The Feulgen-negative plasmosomes also contain neutral mucopolysaccharides and phospholipid-protein complexes (1). Intercommunication of cytoplasm between all members of the 16-cell cyst is made possible by large pores in the walls separating adjacent cells

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(2). At first all 16 germ cells grow at roughly identical rates. However, once vitellogenesis begins the oocyte grows at a rate 10 times faster than previously and at the expense of the nurse cells which shrink and eventually degenerate (3).

Previous studies utilizing autoradiographic methods have shown that at least during stages of active vitellogenesis there is very rapid uptake of RNA precursors such as uridine-H^3 by nurse cell nuclei (4, 5). The rate of cytoplasmic synthesis appears to be very low compared to that of the nuclei of nurse cells. However, the cytoplasm appears to be the main site of protein formation. Earlier work was handicapped by the fact that labeled compounds had to be fed the flies or injected into them. This note will demonstrate that uptake of labeled compounds by isolated ovaries takes place at least over short time intervals.

Three-day-old female *Drosophila melanogaster* belonging to the Oregon-R, wild type strain, were etherized and immersed in *Drosophila* Ringer's solution where the ovaries were removed with jeweler's forceps. The ovaries were transferred to a solution in a depression slide and incubated in the dark at 25°C. The solution consisted of 2 parts TC 199 (6): 1 part uridine-H^3 solution. The radioactivity of the final solution was 15 μc./ml. The ratio between radioactive and non-radioactive uridine molecules was 1 to 44. After incubation periods of 4, 10, or 15 minutes, ovaries were removed and fixed for 1 hour in Caulfield's modification of Palade's fluid (7). The tissue was then dehydrated and embedded in methacrylate (1 butyl: 1 methyl). Sections 5 micra thick were cut using a Leitz-Fernández-Morán microtome and mounted on albumenized slides. The methacrylate was removed with xylene and the slides were rehydrated. Kodak NTB-3 emulsion[1] was liquified in a 50°C. water bath and in complete darkness each slide was dipped in the emulsion, drained vertically, and allowed to gel in a horizontal position for 15 minutes. The slides were dried with a current of air, placed in light-tight, bakelite boxes and allowed to expose for 1 week at 3°C. The slides were then developed (at 21°C.) for 6 minutes in Kodak D 19 developer, washed for 20 seconds in distilled water, fixed for 10 minutes in Kodak acid fixer, cleared for 2 to 3 minutes in Kodak hypo clearing agent, and washed for 5 minutes in a water bath. Subsequently, the slides were dehydrated through an alcohol series to a 1:1 benzene: immersion oil (R.I. 1.46) solution. The preparation was then covered with a glass coverslip which was then ringed with enamel. The autoradiographs were viewed under brightfield and phase contrast optics.

[1] Supplied through the courtesy of Eastman Kodak, Rochester, New York.

Uptake of tritium was demonstrated in the ovaries from the three experimental series. Fig. 1 shows photomicrographs of a portion of the nurse cell chamber of a developing egg in stage 10 (8). Four nurse nuclei are cut in section. The plasmosomes appear as dense blobs within the nuclei. Developed silver grains are concentrated above the plasmosomes. The silver grains appear above molecules (presumably RNA) which were synthesized within 4 minutes from precursors which include exogenous uridine and which after fixation with osmium tetroxide are insoluble in water, ethanol, and methacrylate.

The demonstration of *in vitro* uptake of labeled compounds by the *Drosophila* ovary opens the way to the determination of the actual amounts of exogenous precursor compounds incorporated into nuclear and cytoplasmic structures in the ovary and the effect of antimetabolites in known concentrations upon such syntheses. Studies can also be made of the relative rate of uptake of labeled compounds by ovaries incubated in pairs. Thus the metabolism of an ovary homozygous for a given female sterile mutation can be compared directly with that of a wild type ovary.

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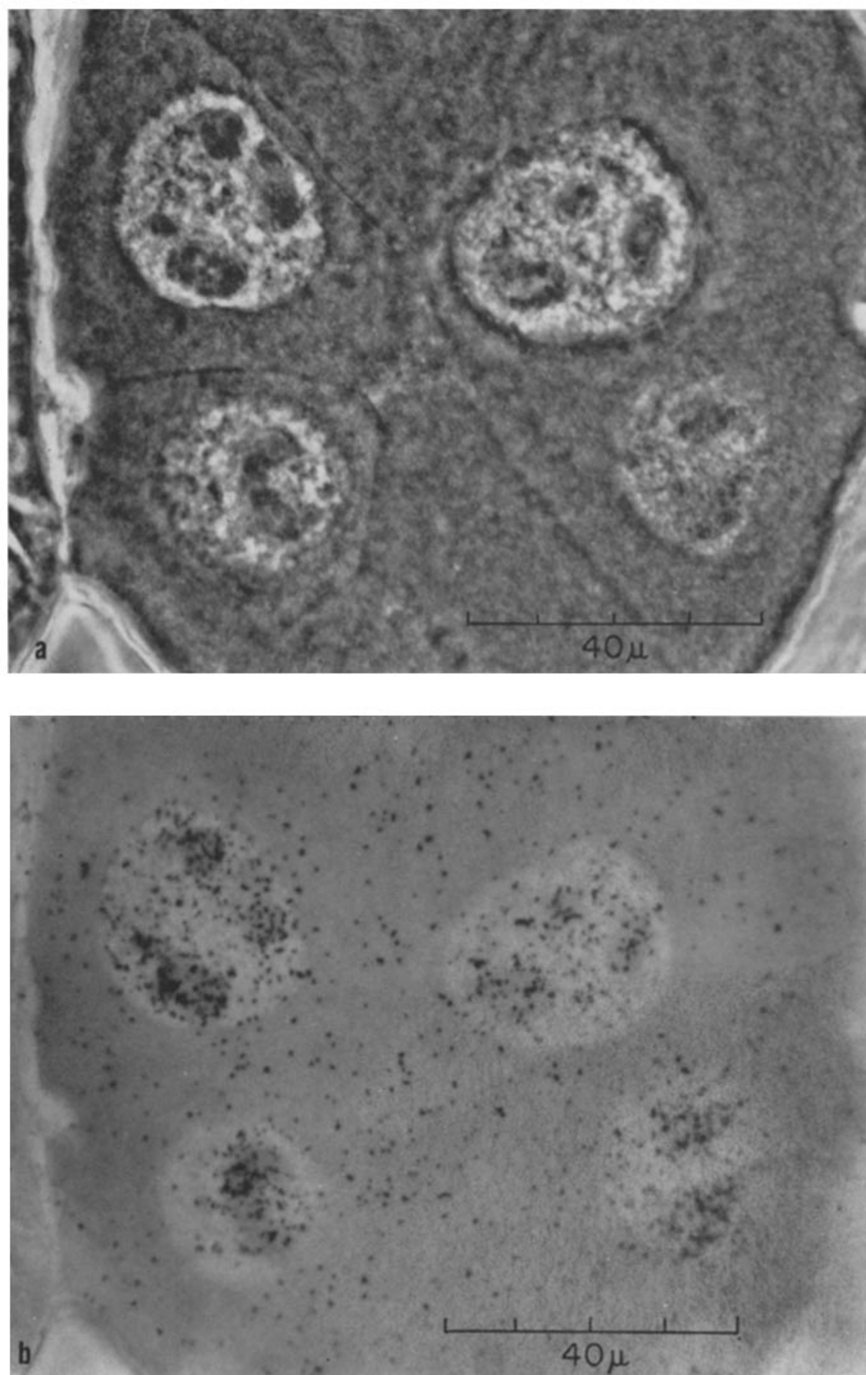

FIGURE 1

(*a*, phase contrast; *b*, brightfield). An autoradiograph of a section through a developing egg from a fruit fly ovary incubated for 4 minutes in a solution containing uridine-H^3.

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