Tung, P.S. Pennsylvania State University, University Park, Pennsylvania. Radioautographic study of RNA and histone syntheses in the testicular cyst cells of Drosophila.

In insects, the germ cells derived from one primary spermatogonium are surrounded by a testicular cyst (Fig. 1 A-B) - a closed envelope of polyvalent cell/cells (Cantacuzene, 1968). Within the cyst, the germ cells develop and differentiate synchronously (Virkki, 1965; Bairati, 1967). As the spermatids elongate, their anterior tips are usually embedded in the cytoplasm of the cyst cells (Fig. 1C). The speculation that the latter are "nutrituve" cells was tentatively based on the morphological association between these two cell types.

In our current studies on the incorporation and interconversion of pyrimidines and of amino acids in Drosophila sperm cells, the testicular cyst cells were found incorporating tritiated uracil throughout the course of spermatogenesis until the time when elongation of the sperm cells is completed (Fig. 1D). This strongly suggests that they are actively involved in RNA and protein syntheses and that their "nutritive" role in the development of the sperm cells surrounded is likely.

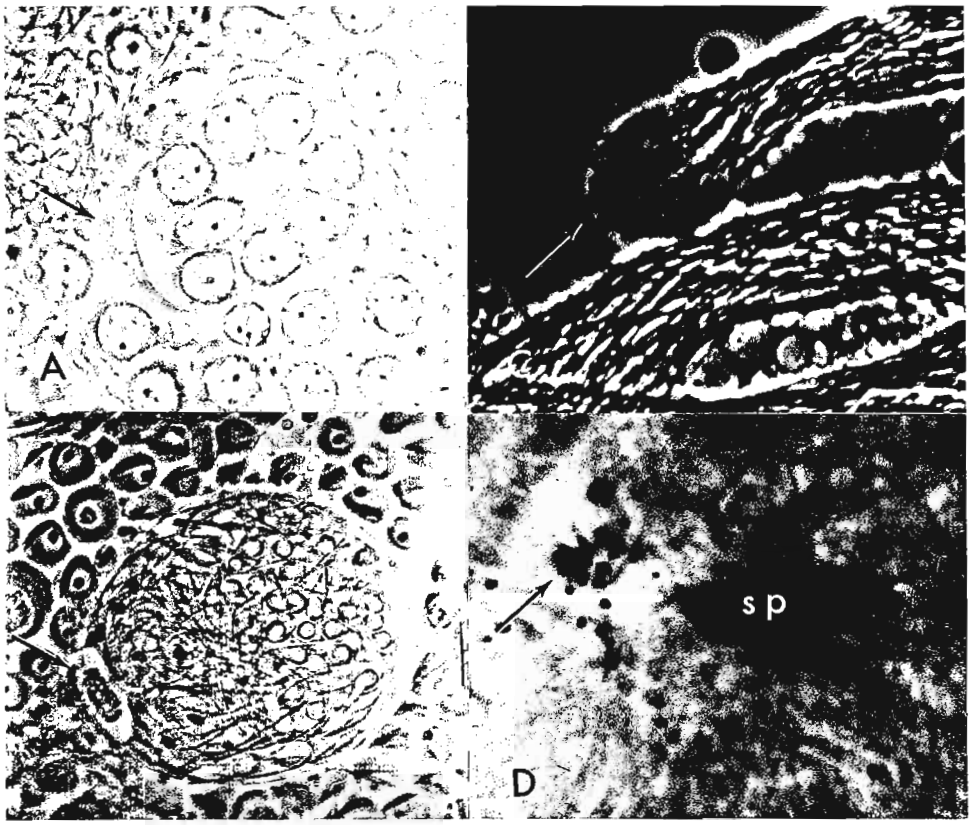

Fig. 1. Testicular cysts at: (A) 8 secondary spermatogonial stage, note cell body of cyst (arrow); (B) early elongation; (C) late elongation, note anterior tips of sperm cells embedded in cyst cell. (D) Radioautograph of cyst cell incorporated with uracil-6-^{3}H, elongation completed. Note bundle of sperm heads (s p).

On the other hand, no significant incorporation of tritiated lysine was evidenced in the cyst cells after elongation of the spermatids began. However, it has been documented that "new" additions of proteins in late spermatids are mainly protamine or arginine-rich histones (Bloch, 1966). One might expect that labeled lysine be actively incorporated during spermiogenesis. The reverse of such an expectation as revealed in the present study supports the hypothesis that histone transition (from somatic-type to gamete-type) during spermiogenesis represents reorganization and re-location of sloughed nuclear proteins (Chevallier, 1966; Vaughn and Thomson, 1972).

However, synthesis of other proteins (e.g. non-basic acrosomal protein and some enzymes) also occurs during spermiogenesis. Since the genome is not transcribing for RNA synthesis as was evidenced in lack of tritiated uracil uptake (unpublished material), the incorporation of tritiated uracil in the cyst cells suggests a possible site of synthesis for these proteins.

In the present study, Drosophila cultures of Oregon R wild type were used. Testes were dissected from 2-day old males in a large drop of Schneider's Drosophila medium (Grand Island Biol. New York). These were rinsed with fresh medium and incubated with isotopically labeled medium. Tritiated uracil used was Uracil-6-^{3}H (sp. act. 26.2 Ci/mM, New England Nuclear, Boston) which was diluted with Schneider's medium to a final activity of 100 μCi/ml and final concentration of 1 x 10^{-6}M. Tritiated lysine used was L-lysine-^{3}H (G) (sp. act. 1.85 Ci/mM, New England Nuclear, Boston) and diluted to a final activity of 10 μCi/ml and a total L-lysine of 2.3 x 10^{-4}M. For each 20 testis pairs, 1 ml of labeled medium was used. After incubation at 25°C for 4 hours, the testes were transferred to unlabeled medium and incubated for another 4 hours. The testes were then rinsed in Shen's saline fixed with aceto-alcohol, and squashed in lacto-carmine. Radioautographes were prepared using NTB-2 photographic emulsion (Kodak, Rochester) and exposed for 4 weeks.

References: Bairati, A. 1967 Z. Zellforsch, Mikrosk. Anat. 79:65; Bloch, D.P. 1966 Protoplasmaforsch 5:1; Cantacuzene, A.M. 1968 Z. Zellforsch, Mikrosk. Anat. 90:133; Chevallier, P. 1966 J. Micros. 5:739; Virkki, N. 1965 Agri. Exp. Rev. Coop. State Res. Serv. USDA.

Tung, P.S. Pennsylvania State University, University Park, Pennsylvania. Dosimetry of Drosophila sperm labeled with tritiated thymidine.

Since the discovery of ^{32}P cell suicide some 20 years ago, interest has increased in the genetic effects produced by radio-isotopes incorporated in animal sperm cells. Unlike procaryotes and cultured cells, the radioactivity of labeled spermatozoa in Drosophila is usually considered difficult to assay due to the complexity of the cell-type and cell-stage conditions of the imaginal testis, and to the extremely small quantity per ejaculation. Without having the rate of disintegration per unit of time per cell (e.g. DPM/cell) specified, to correlate experimental results on a quantitative basis is very difficult.

In the present study, two different approaches of radio-assay for Drosophila sperm incorporated with tritiated thymidine were compared: (1) based on the radioactivity per fertilized egg; and (2) on direct quantitation of mature sperm in seminal vesicles. The former appraoch was based on the findings of Hildreth and Lucchesi (1963) that fertilized eggs of Drosophila are 95% monospermic and 5% dispermic.

In the experiments discussed in this report, Drosophila cultures of Oregon R wild type were used. One-day old males were fed continuously for 48 hours with thymidine-methyl-^{3}H (specific activity 25.2 Ci/mM, New England Nuclear, Boston). The diluent used was a medium similar to Hunt's (1970) except that agar was omitted and the concentration of uridine reduced to 1 x 10^{-5}M. These were mixed to a final activity per unit volume of 0.252 mCi/ml and a concentration of 1 x 10^{-5}M. 0.5 ml of the tritiated medium was given to a group of 10 males according to the feeding technique of Felix (1971). After feeding, the males were allowed to mate to virgin females at a ratio of 1 male to 4 females every 2 days according to the brood technique. Radio-assay was based exclusively on analyses of brood D females and males 6 days after the treating period.

In assay 1, eggs were separated from the regular cornmeal medium on which they were laid with 20% sucrose solution, rinsed with distilled water, and dechorionated with 2.5% sodium hypochlorite. For each sample, about 1,000 eggs were transferred onto 3 x 3 cm sections of filter paper and air-dried in a desiccator. Part of these preparations were extracted with cold (4°C) 2% perchloric acid for 30 minutes to remove any labile, acid soluble precursor materials. Such preparations did not differ markedly from untreated ones in their labelling properties. The egg samples were then burned in an oxygen atmosphere using a tritium oxidizer (Packard Instrument C., Illinois). The vapor thus generated was condensed into water and mixed with a scintillation fluid. Radioactivity thus recovered was counted with a liquid scintillation counter (LS-230, Beckman). The latter was calibrated for every experiment by use of standard tritiated water obtained from the National Bureau of Standards.

In assay 2, an approach based on the seminal vesicles of treated males was undertaken. Seminal vesicles were excised from the adjacent tissues and squashed in Shen's saline. The numbers of sperm in the seminal vesicles were recorded based on quantitation under the phase