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 Since the genome is not transcribing for RNA synthesis as was evidenced in lack of tritiated uracil uptake (unpublished material), the incorporation of tritiated uracil in the cyst cells suggests a possible site of synthesis for these proteins.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

Sample no.	No. of sperm	No. of eggs	DPM	DPM/cell	^{3}HTdR/cell**
1	3868		9050	2.60	
2	3216		8640	2.68	
3	11643		26848	2.31	
4	3084		8750	2.84	
X	21811		53288	10.43	
$\overline{X}$	-		-	2.61	2.61×10^7
5		1000	2898	2.76*	
6		1000	3528	3.36*	
7		1000	3076	2.93*	
8		1000	3172	3.02*	
X		4000	12674	12.07	
$\overline{X}$		-	-	3.02	3.02×10^7

The difference between the 2 means is not significant at 5% level.

* Since the probability of tritium disintegration = 1.02×10^{-7}; disintegration/min-tritium, therefore, 1 disintegration - 1×10^7 ^{3}H

** Modified with a coefficient of 0.957, based on the presumption that 5% of the eggs are dispermic

contrast microscope. The coverslips were removed after freezing on dry ice. Cells attached to the slides and coverslips were rinsed into a graduated test tube with 4% sodium lauryl sulfate and homogenized. Samples of the homogenates were counted with the foregoing scintillation counting technique. The results of these comparisons are in the table.

These results clearly show: (1) that the two techniques are reasonably consistent among samples; and (2) that the fertilized eggs in these experiments gave slightly higher radioactivity counts. However, statistic analyses indicate that such difference is non-significant. This implies that fertilized eggs can be used in the radio-assay of the sperm cells. Since eggs are much easier to manipulate than sperm, the advantage of such an approach is apparent. Further, it may be noted that the wall of the seminal vesicle is comprised of somatic cells. Nevertheless, results from parallel observations on radioautography indicate that these cells do not actively incorporate tritiated thymidine during the treating period. Hence, the presence of these somatic cells in the sample should be acceptable as far as the accuracy of scintillation counting is concerned.

References: Felix, R. 1971 DIS 47:129; Hildreth, P.E. and J.C. Lucchesi 1963 Develop. Biol. 6:262; Hunt, V. 1970 DIS 45:179.

Lefevre, G., Jr. and K. Peterson. San Fernando Valley State College, Northridge California. An unusual Notch mimic: glossy-like (g-1).

In examining an F_2 culture from an EMS-treated + male, we observed viable males that display many of the characteristics of mutations at the Notch (N) locus: strongly notched wings, thickened Confluens-like wing veins, prominent "deltas" at the junctions of the longitudinal veins with the wing margins, extra hairs on the thorax and legs, shortened tarsal joints, and roughened, shiny bright somewhat mottled eyes closely resembling those of facet-glossy (fa^g). In addition, all macrochaetes are thin and delicate. The mutant, however, is not allelic with Notch or any of its alleles; it is, in fact, located less than a map unit to the left of wavy (wy) in Section 11D of the salivary chromosome. Nonetheless, in the presence of Dp w^{+51b7}, which extends from 3C2 through 3D6 and includes the N^+ locus, the phenotypic expression of the mutant, which we have named glossy-like (g-1), becomes virtually normal: small deltas remain, there is some thoracic hairiness, and the eyes are not completely smooth. When raised at 29°, g-1 fails to emerge from the pupal case, and the presence of Dp w^{+51b7} does not protect it from this temperature sensitivity. No chromosomal aberration is present and recombination appears to be normal. When g-1 is in heterozygous combination with a long, male-lethal euchromatic insertional translocation, T(1→3R)C92, having breaks at 6E1-2 and at (or immediately adjacent to) band 11D9-10, the g-1 phenotype is expressed. Both g-1 and wy are uncovered by