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SPERMATIDS RETAIN THE HIGHEST LEVEL OF ETHYLATION IN DROSOPHILA TESTIS AFTER ETHYL METHANESULFONATE OR DIETHYLNITROSAMINE TREATMENT

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Haimei Huang and William R. Lee (1987) Spermatids retain the higest level of ethylation in Drosophila testis after ethyl methanesulfonate or diethylnitrosamine treatment. *Bull. Inst. Zool., Academia Sinica* **26**(4): 305-309. The retained ethylation of 3H-ethyl methanesulfonate (3H-EMS) or 3H-diethyl nitrosamine (3H-DEN) on Drosophila sperm heads was estimated by autoradiography in combination with fluorescent staining technique. The level of silver grain on sperm sampled at various times after mutagen treatment shows that the sperm which originated from the spermatid stage at the time of feeding labeled EMS or DEN had the highest grain count. The mature sperm from 3H-EMS treated samples had the next highest grain count, whereas the mature sperm from 3H-DEN treated samples had the lowest grain count. Digestion with trypsin, pronase and RNase removed 41-67% of ethylation from the sperm heads.

In the germ-line cells of Drosophila testis, spermatid is the most sensitive stage for the sex-linked recessive lethal mutations induced by ethyl methanesulfonate (EMS) or diethyl nitrosamine (DEN) (Fahmy *et al.*, 1957; Vogel *et al.*, 1975). However in sperm stage, while the mutation frequency induced by EMS remains high, that induced by DEN is very low. In this communication, autoradiographic technique was used to study the ethylation of EMS and DEN on the different stages of germ-line cells in Drosophila testis.

MATERIALS AND METHODS

Isotopes

3H-thymidine (3H-TdR, 57 mCi/nmol), 1-3H-ethyl methane-sulfonate (3H-EMS, 4.8 Ci/nmole) and 2-3H-ethyl-diethylnitrosamine (3H-DEN, 3.6 Ci/mmole) were purchased from New England Nuclear, Boston, MA. Labled EMS or thymidine was diluted in 1% sucrose solution. 3H-DEN was diluted in distilled water. The preparation of isotope for feeding flies was the same as described by Aaron and Lee (1978).

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Feeding

1) 3H-EMS or 3H-TdR feeding

Sixty five-day old CB males with a genotype of B Y. bb (KS1+2) y ac/X. Y, InEN2, F were placed in a vial containing a piece of glass filter paper in which diluted 3H-EMS or 3H-TdR was applied (Aaron and Lee, 1978). Twenty-four h later, the flies were transferred to fresh medium. Five flies were sampled at 12 h after isotope feeding. Thereafter five flies were sampled daily until day 10.

2) 3H-DEN feeding

Sixty five-day old CB males were fed with diluted 3H-DEN. After feeding for 24 h, they were then transferred to fresh medium, and six flies were dissected at this hour. In the next 24 h, six flies were dissected at a 6-h interval. The remaining flies were allowed to mate with virgin females to consume the old sperms before they were dissected.

Cytological preparation and autoradiography

Immediately after dissecting, sperm samples from vesicles were placed on a glass slide to make a squashed preparation. In some cases, two vesicles from one fly were transferred to two different glass slides, and two preparations were made. Only one of the two preparations was used for enzyme digestion study. The samples for enzyme digestion were treated first with trypsin (5 mg/ml 1X SSC, Sigma) and RNase (1 mg/ml 1X SSC, Worthington Biochemicals) for 1 h at 37°C, then, pronase (Calbiochem.) was added and digestion was continued for another h. At the end of digestion, the slides were washed with 5% trichloroacetic acid (TCA), 70% ethanol and then with distilled water. For autoradiographic study, the slides were coated with emulsion NTB-2 (Kodak), and exposed for two weeks.

Data analysis

The slides were stained with acridine orange (100 μg/ml phosphate buffer, pH 7.0; Matheson Coleman and Bell) and observed under a Zeiss fluorescent microscope (Holmquist, 1978). The numbers of silver grains in thirty sperm heads per slide were recorded. Only sperm heads in areas of low background (less than three grains per μm^2) (Bisconte, 1979), and clearly separated with other sperm heads were used for silver grain counting. Data were analyzed with SAS program by an IBM 3033 computer.

RESULTS AND DISCUSSION

The silver grains within 30 sperm heads per sample show a random Possion distribution (data not shown) as expected by Perry (1964). Although the average grain counts per sperm head of different-date samples were

TABLE 1

Effect of trypsin, pronase and RNase digestion on the ethylation of sperm head

Feeding	Day of sampling	Grains/sperm head		Grain ratio
		Before digestion	After digestion	After digestion / Before digestion
3H-EMS	1.5	3.51±1.89	1.57±1.43	0.47
3H-EMS	3.5	5.54±2.73	3.25±2.09	0.59
3H-DEN	1-2	0.15±0.37	not done	
3H-DEN	2-5	3.76±2.66	1.45±1.70	0.39
3H-DEN	5-7	2.20±2.76	0.93±1.02	0.42
3H-DEN	7-11	1.93±2.15	0.63±0.82	0.33

a. Total radioactivity for feeding is 3.11 mCi for 3H-EMS, 1.72 mCi for DEN.

b. Average grains from at least three flies with more than 30 sperm samples per fly.

different, the values among flies of the same-date sample were not significantly different.

Sperm samples digested with trypsin, pronase and RNase removed a significant amount of labelling (Table 1). This results indicates that DNA ethylation account only about 1/3 to 1/2 of the total ethylation by EMS or DEN treatment. This result and those of Sega (1976) suggest that ethylation in sperm samples is not only due to ethylation on DNA. Ethylation of protamine in chromatin by ethylating the sulfhydryl groups of cystein was suggested by Sega and (Owen (1978). Table 1 also shows that the enzyme digestion removes more grains from DEN-treated samples than from EMS-treated samples (52% vs. 38%). Similar results were also reported by Sabourin (1981). These results suggest DNA ethylation is more important in the treatment with EMS than with DEN.

Sperm heads giving the highest average

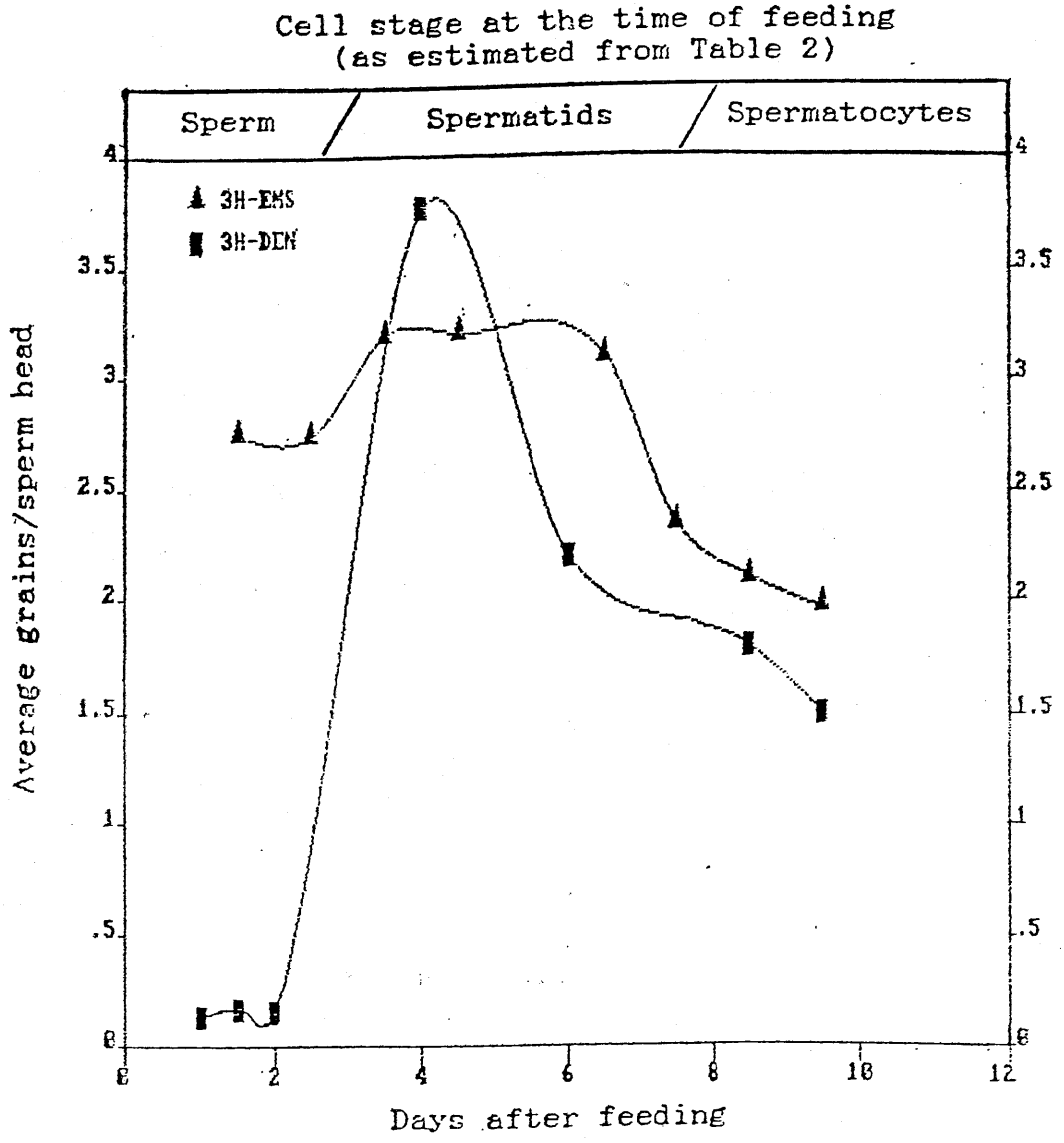

Fig. 1. Labelling of testicular cells after feeding with 3H-EMS or 3H-DEN. (Total radiactivity: 5.18 mCi for 3H-EMS; 1.72 mCi for 3H-DEN.)

TABLE 2
Autoradiographical estimation of the progression of spermiogenesis

	Days after 3H-TdR feeding			
	1-3	4-8	9	10
The most advanced labelling stage	spermatocytes	spermatids	sperm in testis	sperm in the seminal vesicles

grain counts were spermatids at the time of 3H-EMS or 3H-DEN treatment based on the 3H-thymidine estimation of the progression of spermatogenesis (Fig. 1, and Table 2). This result indicates that spermatid is the most sensitive stage for EMS or DEN ethylation. This result agrees with the mutation data of Fahmy and Fahmy (1957), Vogel *et al.*, (1975) and Sobel *et al.*, (1976).

Sperm heads which were mature sperm at the time of 3H-DEN treatment had the lowest average grain counts (Fig. 1). The sperm samples from either 3H-DEN treated male or female (date not shown) all gave very low grain density (<1.5 in overall samples). However, high grain counts remained on mature sperm after 3H-EMS feeding (Fig. 1). Sega *et al.* (1972) also reported that DNA from spermatid at the time of EMS treatment have higher ethylation than DNA isolated from sperm at the time of EMS treatment. These results are also consistent with the mutation data of Fahmy and Fahmy (1959), and Vogel *et al.* (1975). Since the ethylation data match closely with the mutation data, it seems reasonable to assume that the mutations induced by EMS or DEN in Drosophila mainly come from ethylation.

DEN is an indirect alkylating agent that requires microsomal enzymes for ethylation (Vogel *et al.*, 1975). Whereas EMS can act directly without enzyme activation. In Drosophila, endoplasmic reticulum which contain the microsomal enzymes was not found in mature sperm (Tates, 1971). This may explain why low grain counts in DEN-treated sperm heads and high grain counts in EMS-treated sperm heads.

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乙基磺化甲烷或二乙基亞硝胺在雄性果蠅生殖細胞中之乙基殘留量以未成熟之精子爲最高

黃海美　李威連

果蠅雄蟲經含放射性乙基的致變劑——乙基磺化甲烷或二乙基亞硝胺處理後，不同時間所收集的成熟精子樣品中，乙基之殘留量用自動放射顯相技術所獲得之銀粒子數目來代表。結果發現：銀粒子數目最多的精子樣品是來自於致變劑處理時爲不成熟的精子細胞。放射性乙基甲烷處理過的成熟精子，銀粒子含量很高；但經放射性二乙基亞硝胺處理過的成熟精子，銀粒子含量極低。胰蛋白酶、蛋白質分解酶以及核醣核酸切除酶聯合作用可以移去大約 41～67%的放射性乙基。