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Effect of Malathion on Sperm Morphology of Mice

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ABSTRACT

This study dealt with the determination of the effect of Malathion on the sperm morphology of mice. Male mice were treated intraperitoneally and orally with doses of 115 mg/kg and 460 mg/kg body weight. The former induced more sperm morphology abnormalities than the latter treatment. Abnormalities on the acrosomal/head morphology and incidence of headless sperms were analysed through smear preparation. The incidence of variant abnormal shapes of the acrosome was found to be dose dependent with almost 4-fold increase, whereas frequency of headless sperms was age dependent. The types of abnormalities were observed in the following order of decreasing incidence: amorphous > rhomboid > beak > balloon > distally branched > banana > funnel > sunflower > calyx branched > double headed. Changes in the head shape may be correlated to changes in the motility and penetrating capacity of the sperms, with balloon type as the most critical because of absence of hook which is vital for the entry of sperm to egg.

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INTRODUCTION

Researchers are very much concerned with anticipated problems posed by the use of chemical pesticides, i.e. insecticides, molluscicides and herbicides, not only in agricultural countries like the Philippines but also elsewhere in the world. Man himself is inevitably exposed to these chemicals during manufacture, formulation, and application, posing such dangers to the farmer, especially as chronic toxicity of residues and fatal poisoning. Global demand for grain and grain products requires a complex system of transport, storage and processing before final distribution to the consumers. To protect these products, licensed specialists routinely apply fumigants and pesticides, thereby exposing themselves to health hazards (1). Concern over the potential mutagenicity of organophosphates increased when trimethyl phosphate, a simple organophosphate, was found in 1970 to be mutagenic to mice (2).

Malathion, [0,0-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate], is a pesticide commonly used in the Philippines. Although it is one of the least toxic of the commercial organophosphorous insecticides, it is known to contain several impurities such as isomalathions (0,0,0-trimethyl phosphorothioate, 0,0, S-trimethyl phosphorothioate and 0, S, S-trimethyl trimethyl phosphorodithioate) that potentiate its toxicity because they are themselves quite toxic. Studies have shown that 0, 0, S-trimethyl phosphorothioate (0,0,S-Me) binds covalently to pulmonary tissue macromolecules; this raises the possibility that the pneumotoxicant is also an alkylating agent and a mutagenic substance (3). Studies done by Dulout and co-workers reveal that malathion also has the ability to increase the frequency of micronuclei and chromatin bridges in the anaphase-telophase stages in mice, which is indicative of its clastogenic capacity (4, 5, 6). Malathion was also found to induce sister-chromatid exchanges and cell cycle delay (7, 8). Increment in chromosomal aberrations were likewise detected in human beings acutely intoxicated with this compound (2, 9). The potential toxicity of this pesticide can be measured by sperm-morphology assay, i.e. a change in the morphology of the sperm. This assay, developed by Wyrobek to detect mutagenic compounds, has been used to evaluate the potential testicular toxicity of pesticides on mice (10, 11).

The mouse was chosen as an experimental model because of the many similarities of its spermatogenesis to that of man (12).This study determined 1) the potential testicular toxicity of malathion on mice; (2)

the dose level that may induce greater sperm abnormalities; and (3) the relationship between sperm morphology and fertility; (4) identified and classified sperm abnormalities; and (5) determined which route of exposure was more effective.

The relevance of this study stems from the fact that pesticides are potential mutagens and man is most likely to be affected both directly and indirectly. Thus, measures should be taken to minimize the extensive use of pesticides. Through this study, it is hoped that users will be discouraged from using them, and opt instead to use biological means of controlling pests. The findings from this work may provide additional information to the field of genetics and physiology. Sperm abnormalities may serve as indicators of the effect of this pesticide, which may lead to sterility or death.

MATERIALS AND METHODS

A. Test organism

Seven to fifteen week old ICR strain male mice, weighing 12.96–28.30 g, purchased from the College of Veterinary Medicine Hospital, Diliman, Quezon City, were used in this experiment. They were acclimated for one week in the laboratory at the Natural Sciences Research Institute. Two to three preliminary treatments were done before the final experiments.

B. Application of Malathion

A high dose (460 mg/kg per body weight) and a low dose (115 mg/kg per body weight) of Malathion (commercially available at 570 g/l) were used, diluted with 0.25 ml distilled water. These were introduced to the male mice by intraperitoneal (i.p.) and oral routes. One male mouse was used per treatment. The control mouse for intraperitoneal treatment was injected i.p. with 0.25 ml of distilled water, while the control mouse for oral treatment was given 0.25 ml of distilled water orally from a needleless syringe.

C. Sperm-Morphology Assay

Thirty five (35) days after the introduction of malathion, all mice were killed by cervical dislocation. The abdomen was cut-open immediately (Fig. 3), and the cauda epididymis was removed and trimmed of connective tissue. The isolated tissue was placed in a petri dish containing 4 ml physiological saline and minced with scissors. A suspension was mixed by repipeting and then filtering through 80μ silk cloth. Five to seven glass slides were used with a drop of the suspension smear per slide. These were air dried, then fixed in absolute methanol for 10 mins. Staining was done in 10% Giemsa for one hour. One thousand sperms per animal were examined, classified and scored (with a tally counter) as to the shape of the acrosome. Sperm abnormalities were categorized and scored visually according to the criteria of Wyrobeck and Brice (1975) cf. (13) as follows: calyx type, amorphous, balloon type, sunflower type, double headed, branched type, distally branched type, rhomboid type, banana type, funnel type and beak type acrosomal end. Pictures of the slides with sperms were taken using a Zeiss Microscope with camera attachment.

This procedure was based on the work done by Osterloh et al. (10) and by Bhunya and Pati (11), with slight modifications.

RESULTS AND DISCUSSION

Weights of the mice and the corresponding dose volumes administered are presented in Table 1. The frequencies of abnormal sperm heads induced by Malathion are seen in Table 2. Only 1.6% frequency of abnormal sperms was observed in the control mouse, but in the 115 mg/kg group, 5.6% and 4.3% frequencies of abnormal sperms were observed in mice treated intraperitoneally and orally, respectively. These values approximate three-fold and two-fold increases, respectively, from the control. The presence of abnormal sperms in control mice is also reported in studies using this assay (10).

The highest frequency of abnormalities was observed in the mouse treated intraperitoneally with 460 mg/kg Malathion, which gave a value of 8.9% or about a four-fold increase. However, in the mouse treated orally with 460 mg/kg Malathion, the increase in incidence of sperm abnormalities was only 5.6%, three-fold like that treated i.p. with 115 dose. These findings indicate dose dependence: the higher the dose, the greater the frequency of sperm abnormality. The greater frequency of abnormality with higher dose is due to the increased availability of the chemical in the target tissue or cells. This was also noted by Bhunya and Pati (11) in an experiment using copper sulphate. As to the mode of administering the chemical, i.p. is more damaging to the sperm because the dose is better controlled and better directed to the target tissue. In oral administration, the pesticide passes through the digestive system, thereby spreading its toxicity wider and leaving less for the target. Some chemicals are known to be absorbed with difficulty in minute quantities from mucous surfaces. This is another plausible reason for the smaller effect if administration is via the oral route (11).

The unique hook head shape of the sperm in mouse makes variant abnormal shapes easy to recognize (10). Wyrobek et al. (14) showed in limited timing studies with other compounds that mutational damage occurs at the spermatid stage and is best assessed when the spermatids have reached the cauda epididymis around day 35. Large increases in sperm abnormal shapes are associated with reduced fertility. A number of lines of evidence suggest that induced changes in sperm morphology reflect genetic damage in the male germ cell (15). The types of sperm abnormalities and their incidence in terms of treatment are listed in Table 2. These types are as follows in terms of decreasing incidence: amorphous > rhomboid > beak type > balloon > distally branched > banana > funnel > sunflower > calyx type > branched > double headed (Figs. 1–21). The shape of the head/acrosome of sperms may be correlated to their penetrating capability. Abnormalities in the sperm could affect their fertilizing ability/chances of successfully penetrating the egg. This was proven by Meistrich and co-workers (16) using Adriamycin. They reported that the induced sperm abnormalities subsequently reduced the fertility of the mice. The amorphous type was the most frequently observed abnormality in the treated mice. This form lessens the motility of the sperm because its enlarged head decreases the chances of reaching the egg. Balloon conformation appears to be the most critical among the abnormalities observed. Such sperms lack the hook which is vital to penetration. Funnel, sunflower, calyx and double headed conformations are also critical, but they are low in frequency. They pose minimal effect on fertilization in malathion-affected mice. Other sperm abnormalities such as rhomboid, beak, distally branched, and branched types may inhibit the motility of the sperms, on the other hand, the banana type may even enhance sperm movement because of the slender head shape with hook.

Although the exact mechanism by which sperm abnormalities are induced by mutagens is not known, the abnormal morphology is believed to be transmitted for at least two generations, and increases in the frequency of abnormal sperms are thought to be a consequence of either point mutations or small deletions (17). Malathion, an organophosphorus insecticide, is known to be an alkylating agent that interferes with the nucleophilic sites of the DNA (3, 18). Seven-methylguanine was found to be an abundant product of DNA methylation, as observed in salmon sperm DNA, *E. coli*, and mice which were made to react with methyl 14C-labelled dichlorvos, an organophosphorus insecticide. Other alkylation products were 1-methyladenine, 3-methyladenine, 3-methylguanine, 6-0-methylguanine and 3-methylcytosine. High concentrations of dichlorvos produced non-random disintegration of DNA molecules rather than random strand breakage (2). Considering that treatment with Malathion induced a four-fold increase in sperm abnormalities in this study, it can be stated that this pesticide is mutagenic. This abnormality manifested in the head which bears the genetic material could have caused the mutagenic effect.

The incidence of headless sperm (head detached) in mice, as presented in Table 3, is possibly influenced by age and not by dose as evidenced by a lower percentage of headless sperms in 460 i.p. treatment compared to 115 i.p. treatment. Mice aged 13 and 15 weeks treated with 115 dose (i.p.) and 460 dose (oral), respectively, had higher frequencies of headless sperms than 7 to 12 week-old mice given the same treatments. Further study is indicated to assess the correlation between mice age and Malathion dosage in production of headless sperms.

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Table 1. Experimental Set-up for Sperm Morphology Assay using Malathion

DOSE	INTRAPERITONEAL		ORAL	
	WEIGHT	DOSE VOL.	WEIGHT	DOSE VOL.
115 mg/l	23.30 g	4.7 ul	12.96 g	2.61 ul
460 mg/l	20.17 g	16.28 ul	28.30 g	22.8 ul
Control	21.97 g	0.00 ul	22.07 g	0.00 ul

Table 2. Frequency of Abnormal Sperms Induced by Malathion

Type*	Control	115 IP	115 Oral	460 IP	460 Oral
A	2.00	2.00	0.00	0.00	0.00
B	4.00	5.00	9.00	25.00	29.00
C	0.00	11.00	0.00	18.00	1.00
D	0.00	2.00	0.00	1.00	4.00
E	0.00	0.00	0.00	0.00	1.00
F	0.00	1.00	0.00	2.00	1.00
G	0.00	13.00	8.00	2.00	6.00
H	0.00	10.00	6.00	13.00	5.00
I	0.00	6.00	4.00	9.00	7.00
J	1.00	2.00	1.00	7.00	2.00
K	7.00	4.00	15.00	12.00	0.00
SUM	16.0	56.00	43.00	89.00	56.00
%	1.6	5.6	4.3	8.9	5.6

N = 1000
Legend: * Types of sperm abnormalities

A calyx
B amorphous
C balloon
D sunflower
E double-headed
F branched
G distally-branched
H rhomboid
I banana
J funnel
K beak

Table 3. Frequency of Headless Sperms in Mice

DOSE (mg/kg)	INTACT HEAD	HEADLESS SPERMS	FREQUENCY %
control	987	13	1.3
115 IP	845	155	15.5
115 Oral	952	48	4.8
460 IP	946	54	5.4
460 Oral	868	132	13.2

Note: Frequency = headless sperm/(intact head + headless sperm)

Fig. 1. Control sperms observed under the light microscope (X437) some of which have detached head/headless (**H**)*. Notice the unique hook shape of the acrosome* (**hk**)*.*

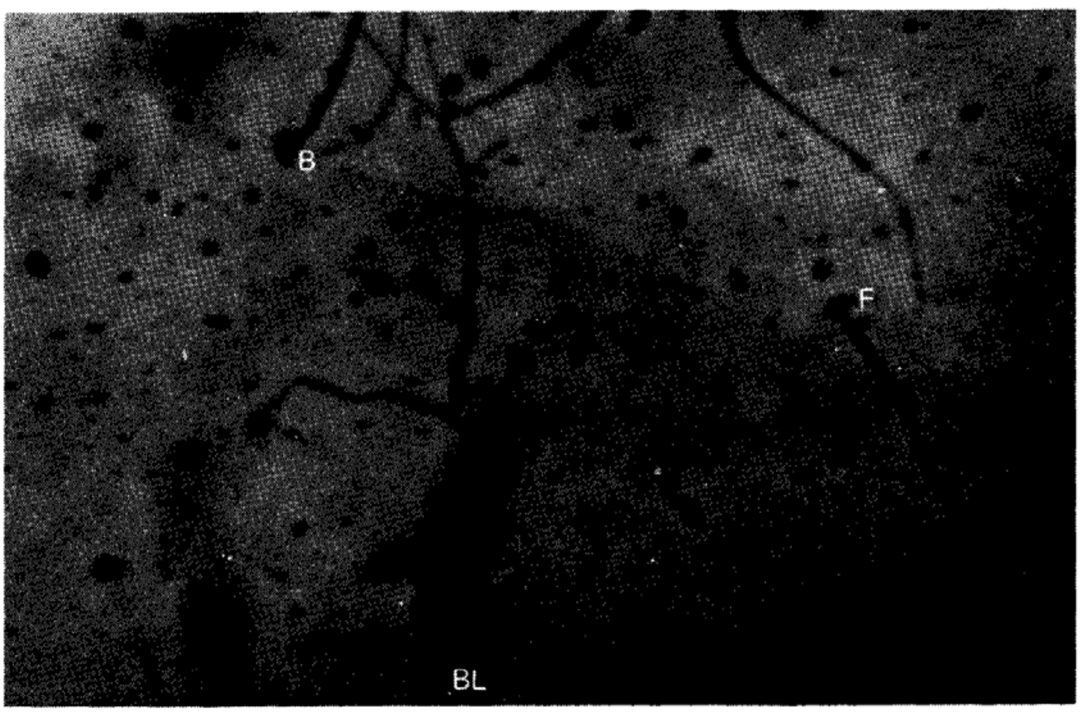

Fig. 2. Control mouse sperms with some abnormalities like beak (**B**)*, funnel* (**F**)*, and balloon* (**Bl**) *observed under the light microscope (X437).*

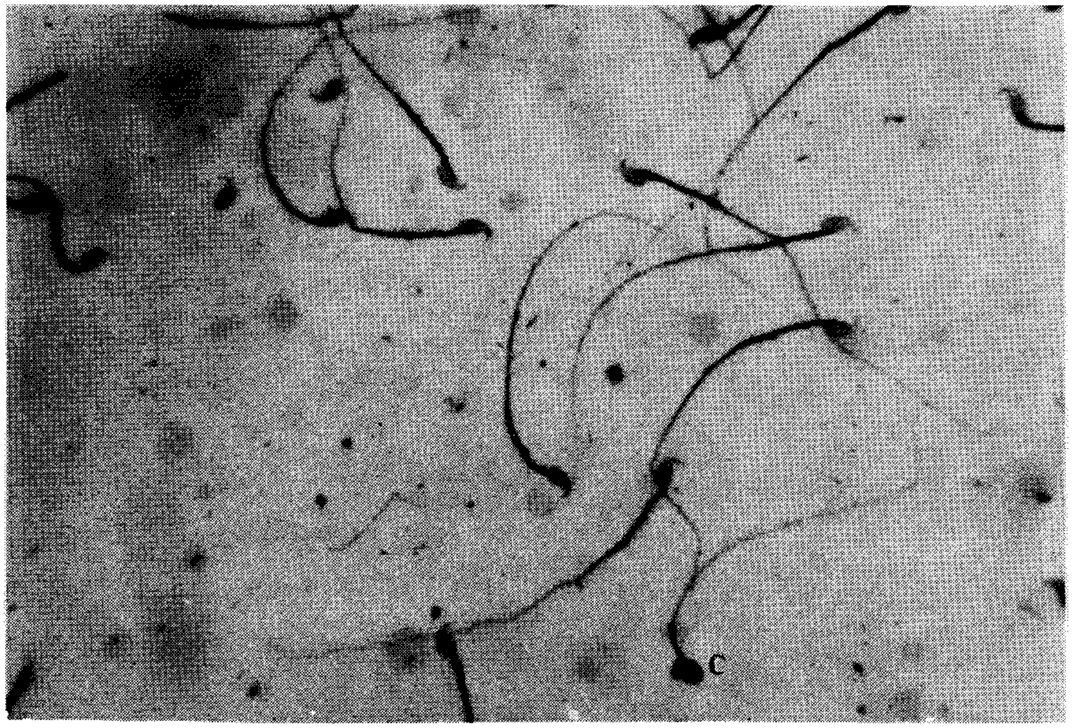

Fig. 3. Control mouse sperm with calyx (**C**) *type of abnormality observed under the light microscope (X437).*

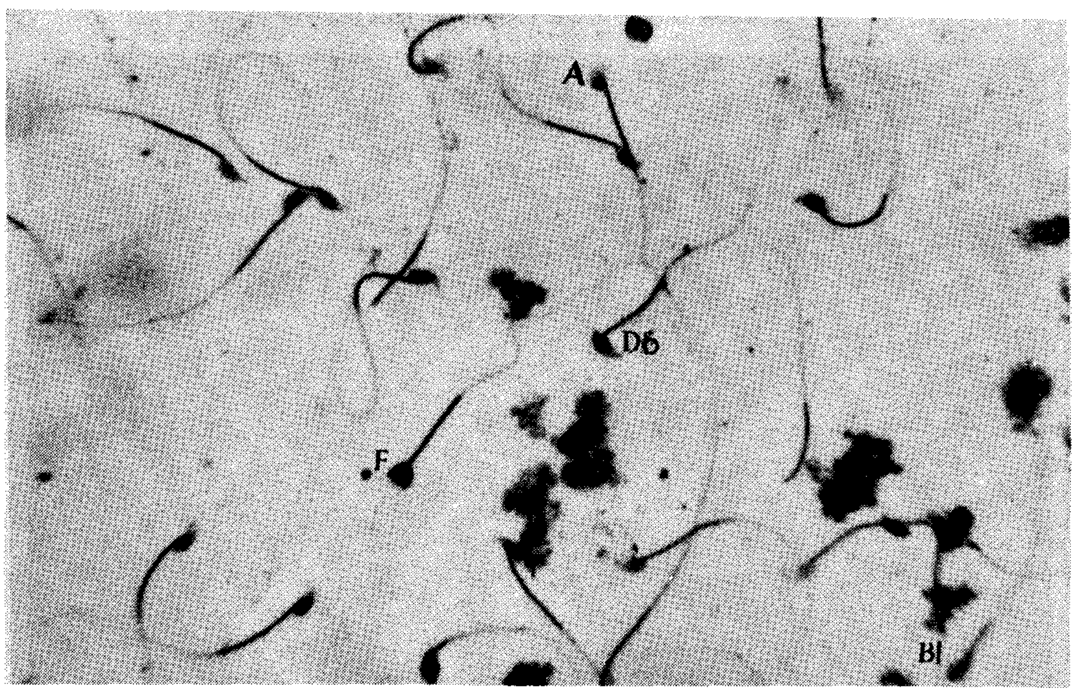

Fig. 4. Sperms obtained from mouse treated intraperitoneally with 115 mg/kg dose of Malathion as observed under the light microscope (X437). The abnormalities were: funnel (**F**), *balloon* (**Bl**), *distally branched* (**Db**), *and amorphous* (**A**).

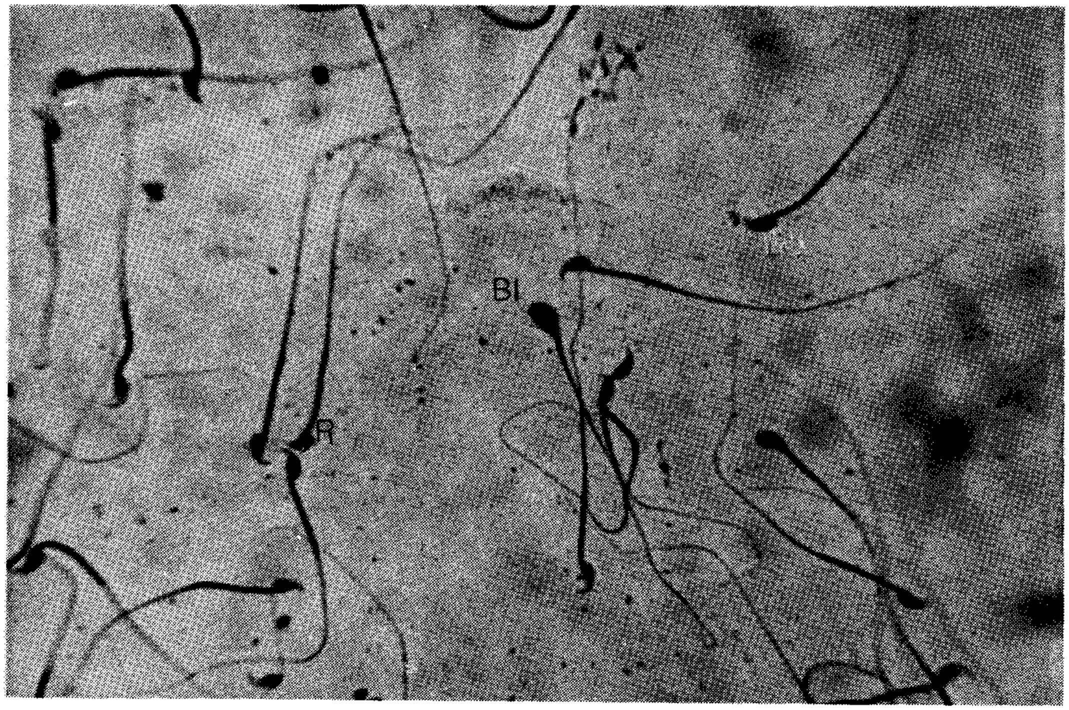

*Fig. 5. Sperms taken from mouse treated intraperitoneally with 115 mg/kg dose of Malathion as observed under the light microscope (X437). The abnormalities were: balloon (**Bl**) and rhomboid (**R**).*

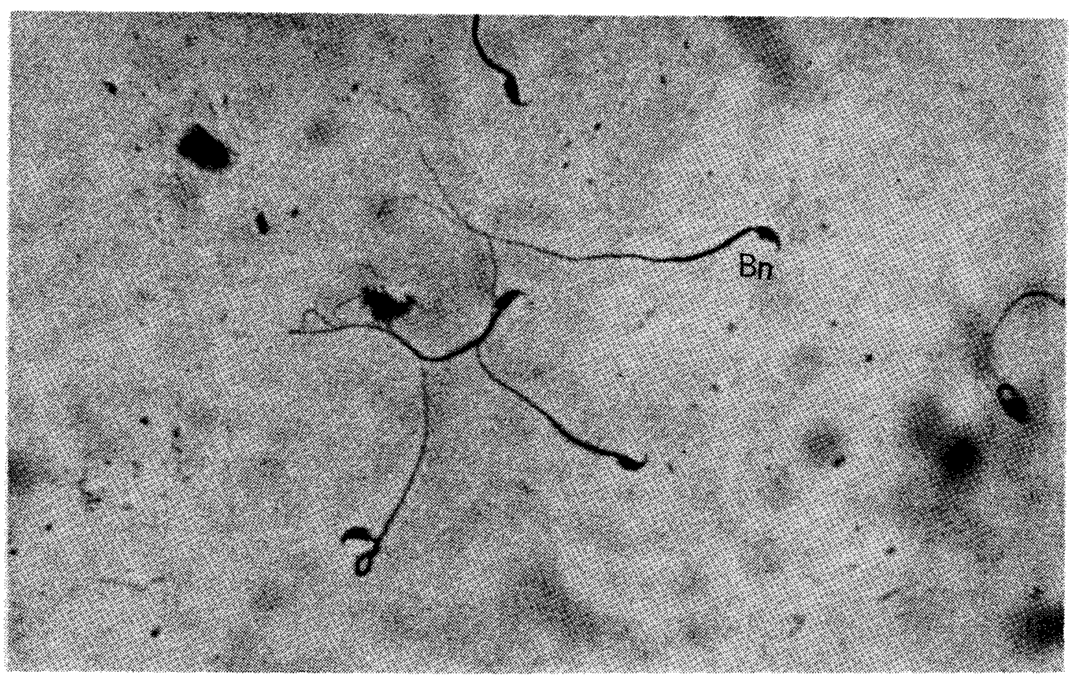

*Fig. 6. Sperms observed from mouse treated intraperitoneally with 115 mg/kg dose of Malathion under the light microscope (X437). The abnormality was banana type (**Bn**).*

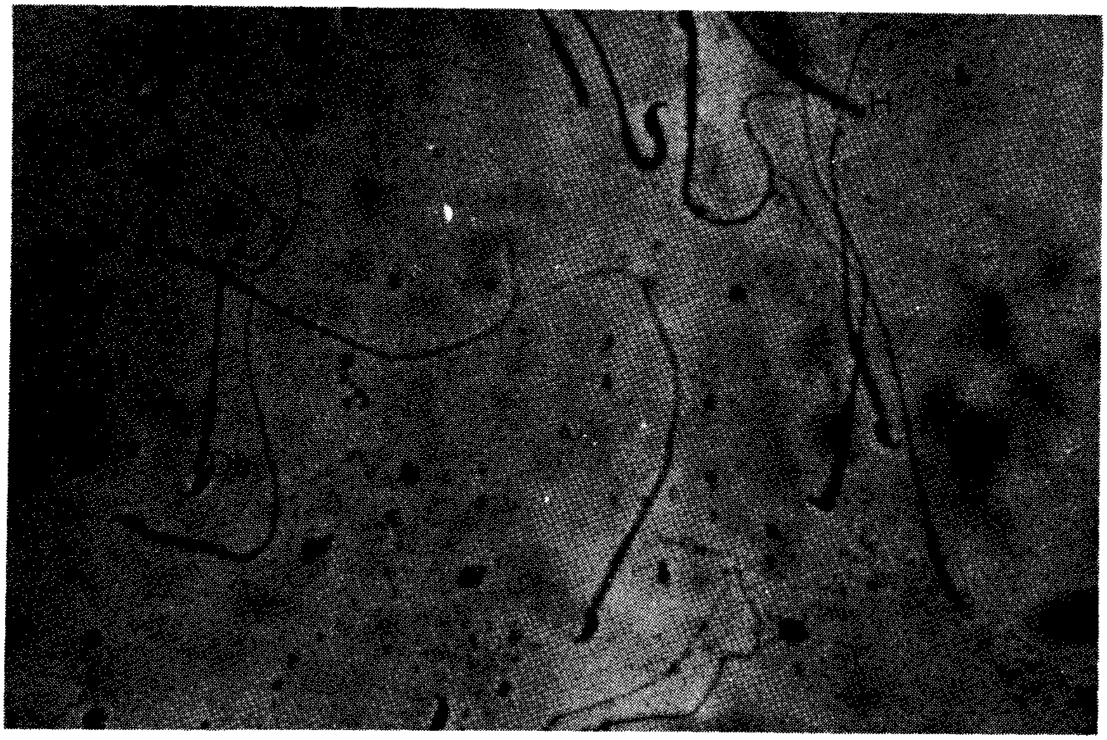

Fig. 7. Sperms obtained from mouse treated intraperitoneally with 115 mg/kg dose of Malathion. Notice the headless sperms (**H**) *and distally branched* (**Db**) *sperm observed under the light microscope.*

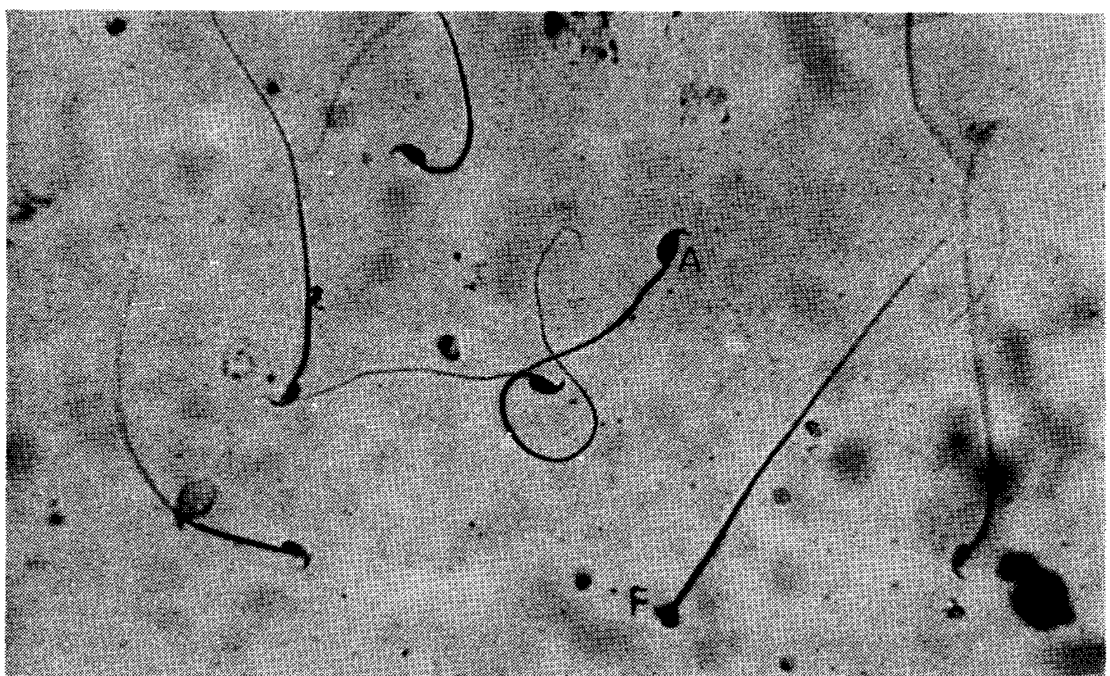

Fig. 8. Sperms observed from the mouse administered orally with 115 mg/kg dose of Malathion as seen under the light microscope (X437). The abnormalities were: amorphous (**A**) *and funnel* (**F**).

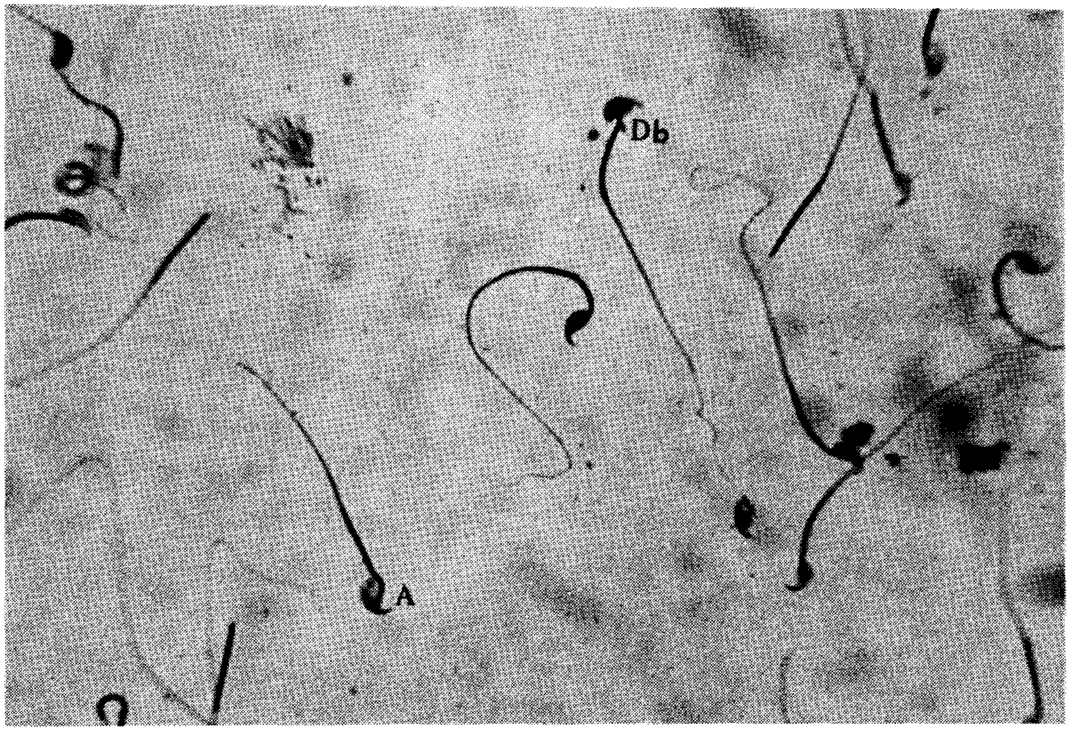

Fig. 9. Sperms obtained from mouse administered orally with 115 mg/kg dose of Malathion. Notice the amorphous **(A)** *and distally branched* **(Db)** *types of abnormalities as observed under the light microscope (X437).*

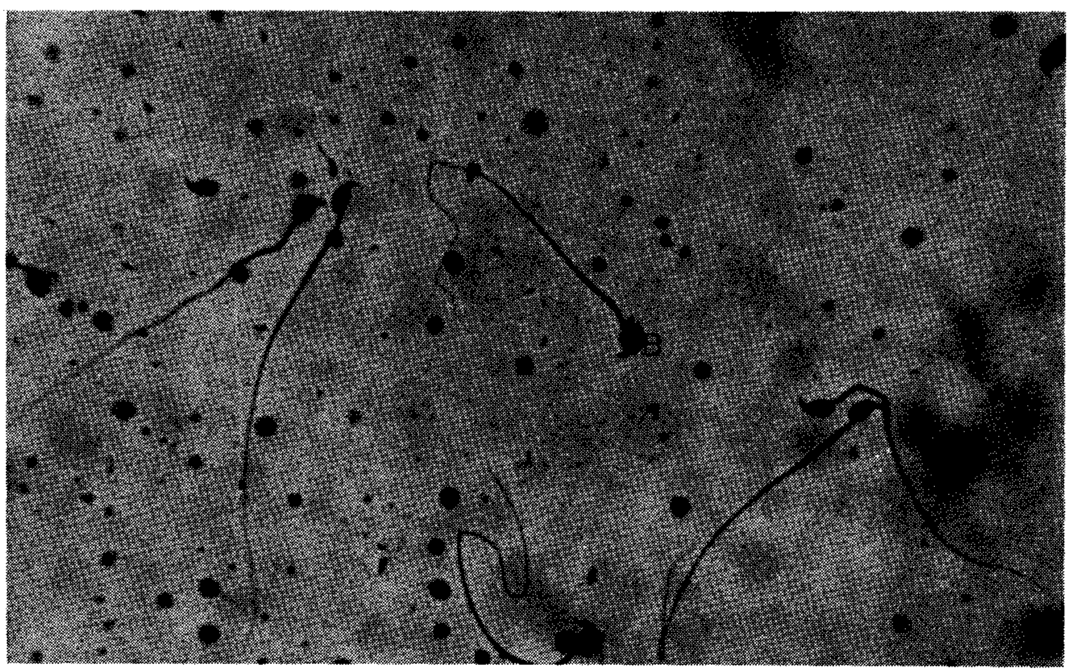

Fig. 10. Sperms obtained from mouse administered orally with 115 mg/kg dose of Malathion. The field consists of numerous beak type **(B)** *of abnormality observed under the light microscope (X437).*

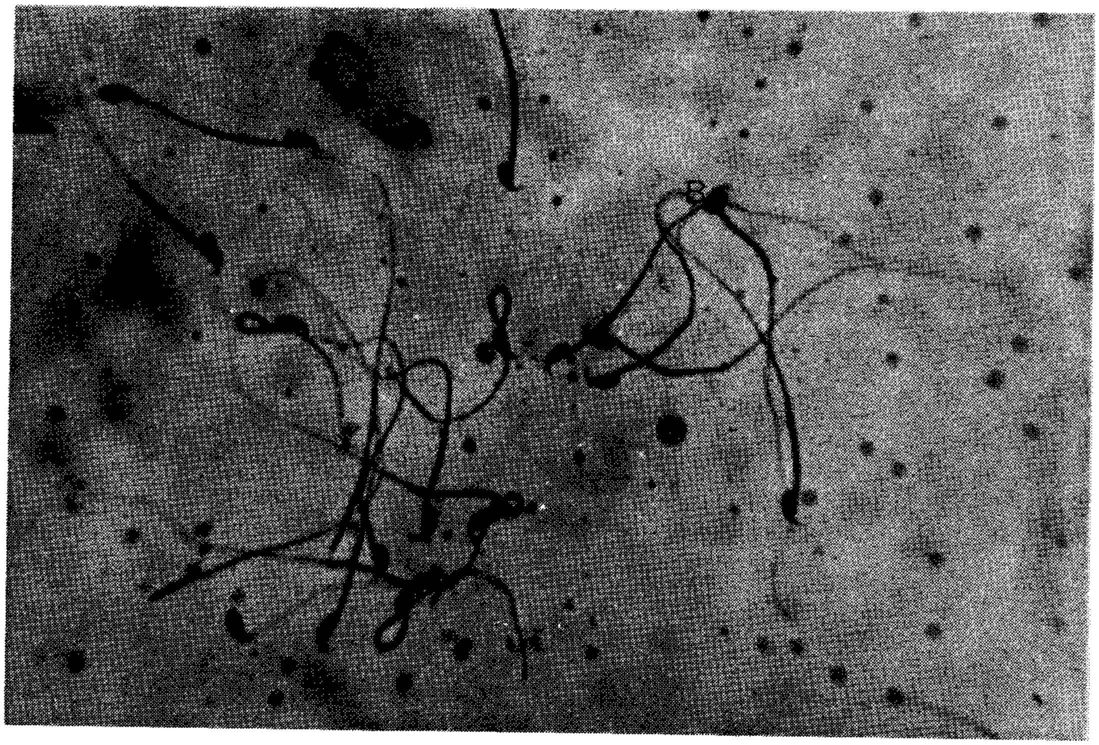

Fig. 11. Sperms observed from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. Notice the beak (**B**) *type of abnormality seen under light microscope (X437).*

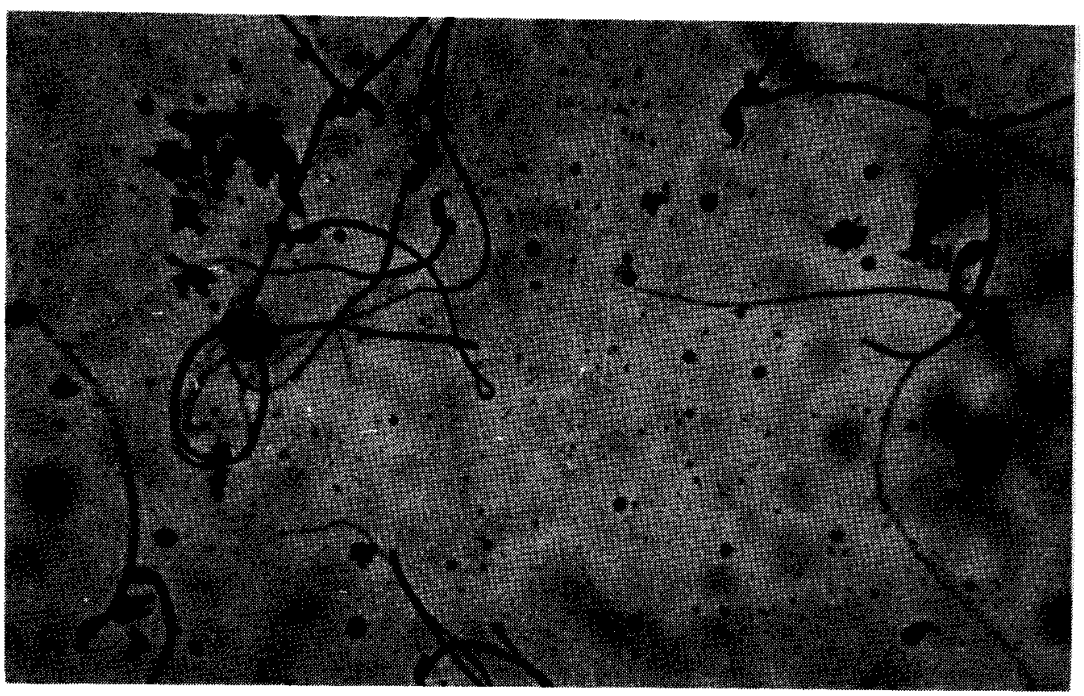

Fig. 12. Sperms observed from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. The abnormalities observed were: beak (**B**) *and balloon* (**Bl**) *under the light microscope (X437).*

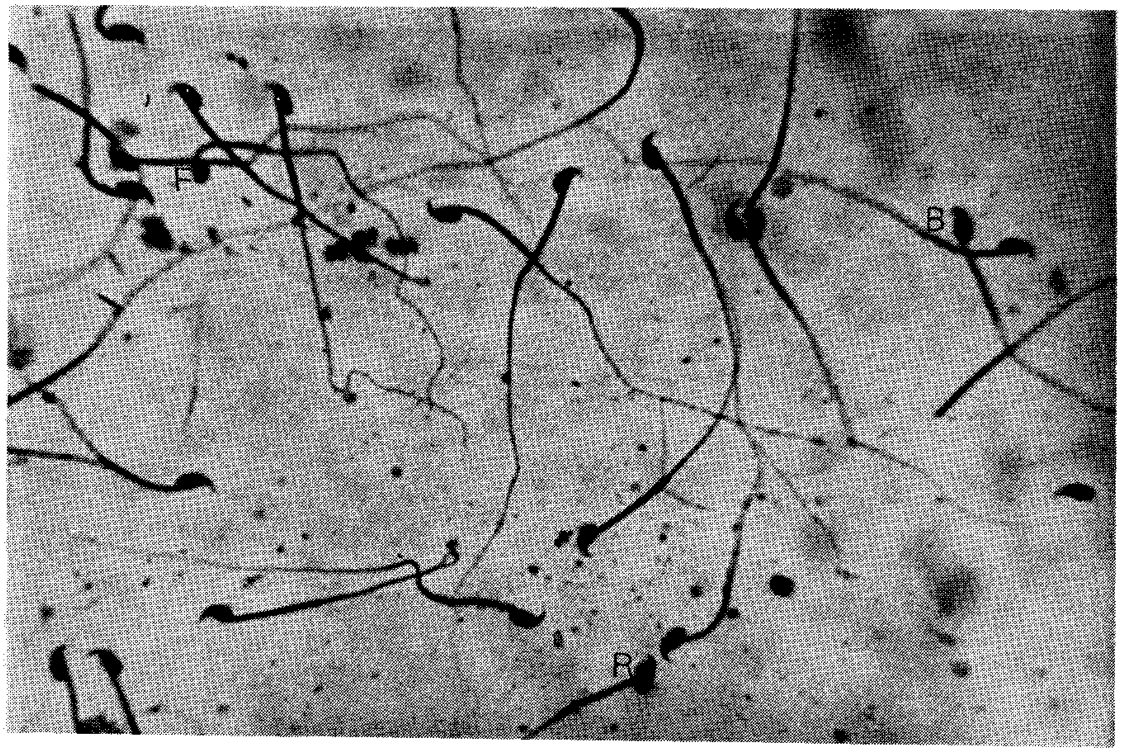

Fig. 13. Sperms observed from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. The abnormalities observed were: beak (**B**)*, rhomboid* (**R**)*, and funnel* (**F**) *under the light microscope (X437).*

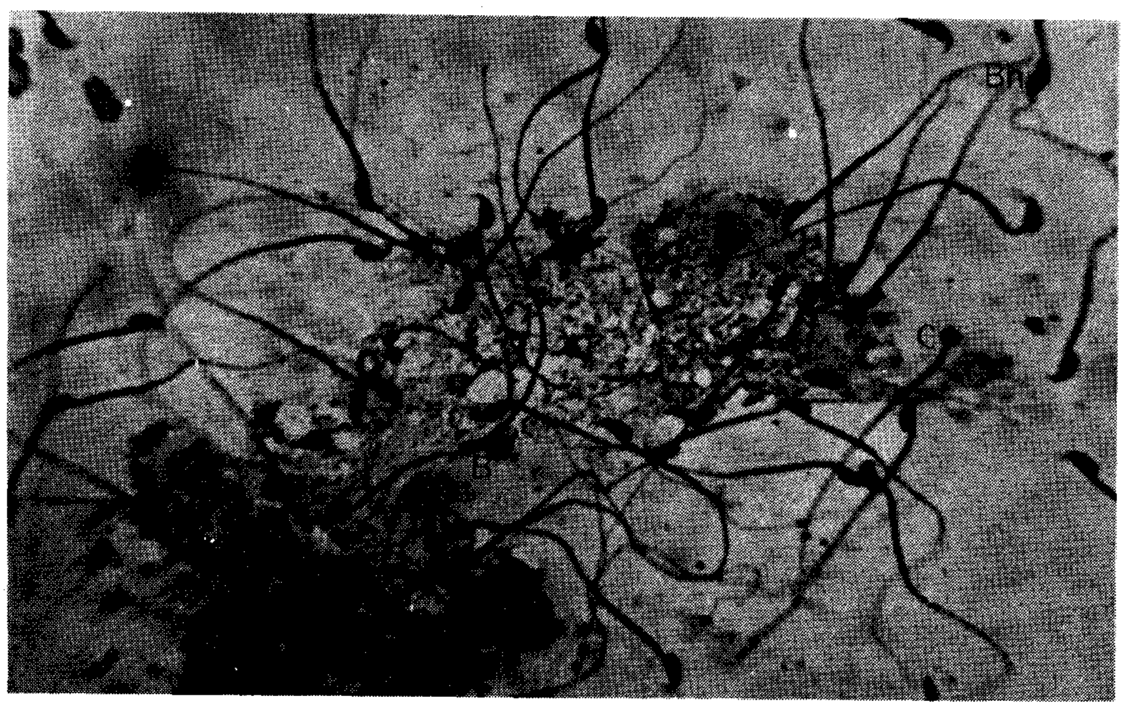

Fig. 14. Sperms observed from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. The abnormalities observed were: banana (**Bn**)*, calyx* (**C**)*, and beak* (**B**) *as seen under the light microscope (X437).*

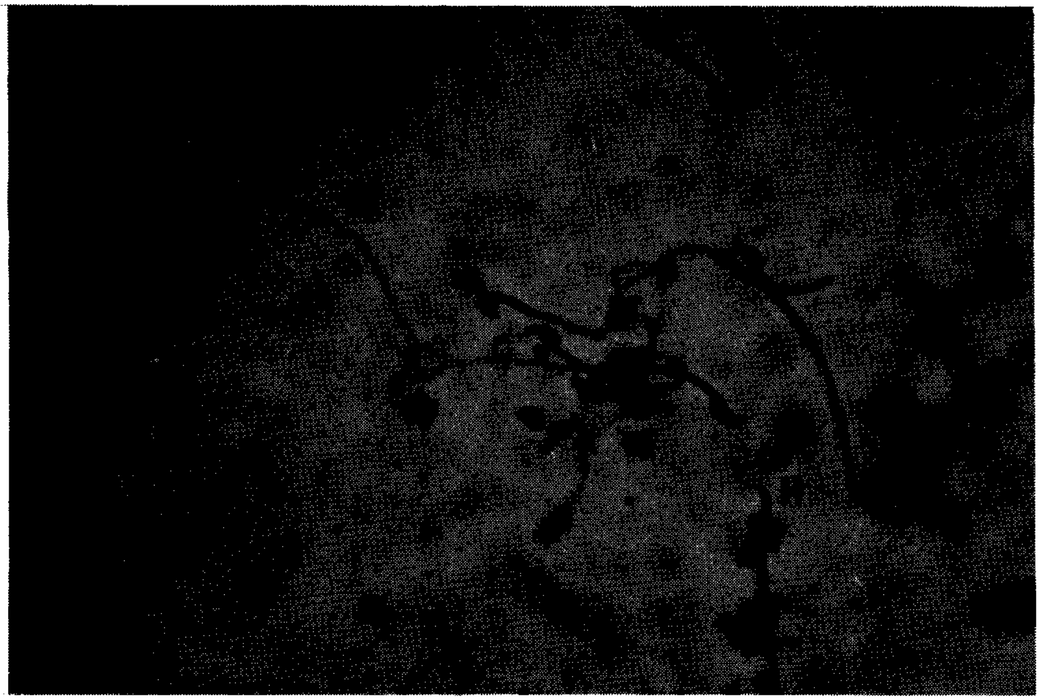

Fig. 15. Sperms taken from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. Notice the sunflower type (**S**) *of abnormality and the headless sperm* (**H**) *as observed under the light microscope (X437).*

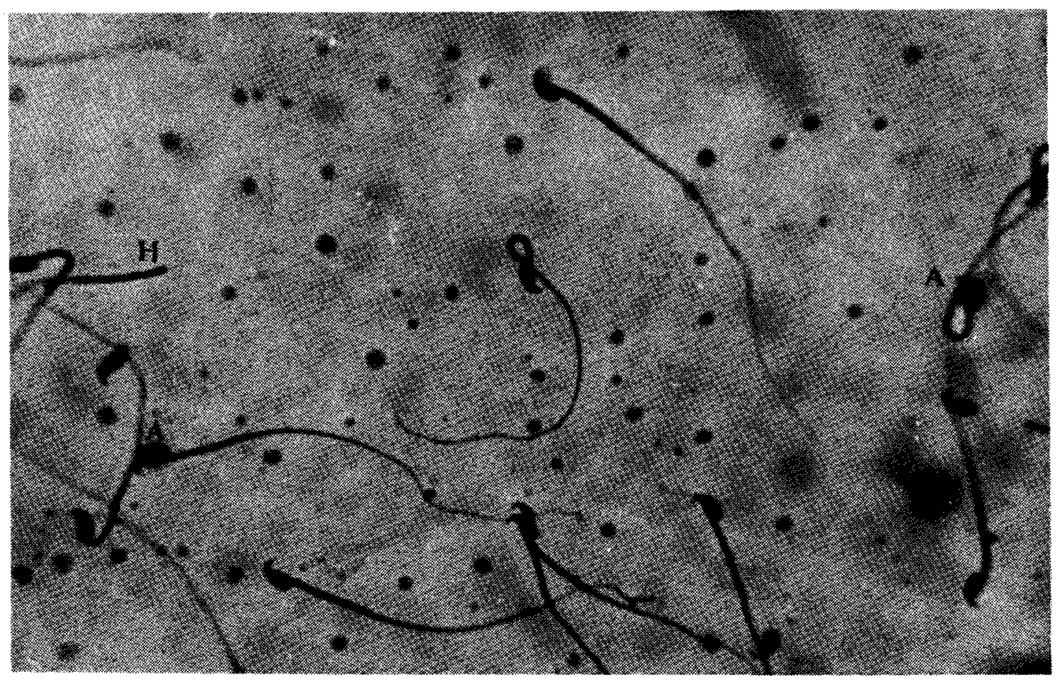

Fig. 16. Sperms obtained from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. Notice the amorphous type (**A**) *of abnormality observed under the light microscope (X437) and the presence of headless sperms* (**H**).

Fig. 17. Sperms obtained from mouse treated intraperitoneally with 460 mg/kg dose of Malathion. Notice the branched type of abnormality observed under the light microscope (X437).

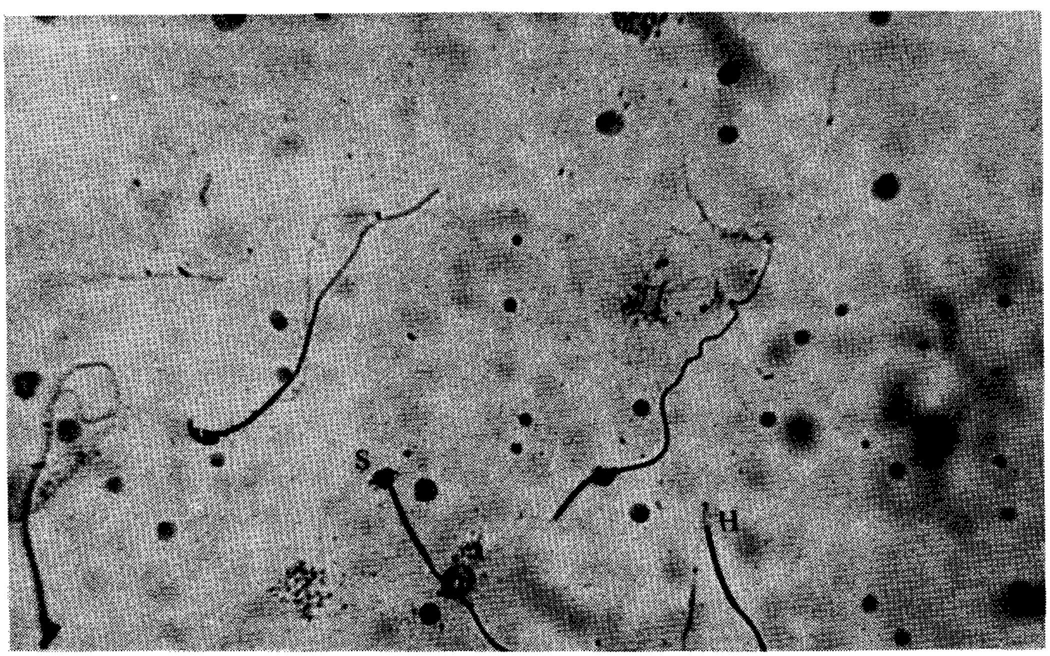

Fig. 18. Sperms obtained from mouse treated orally with 460 mg/kg dose of Malathion. Notice the sunflower type (S) of abnormality and the presence of headless sperm (H) observed under light microscope (X437).

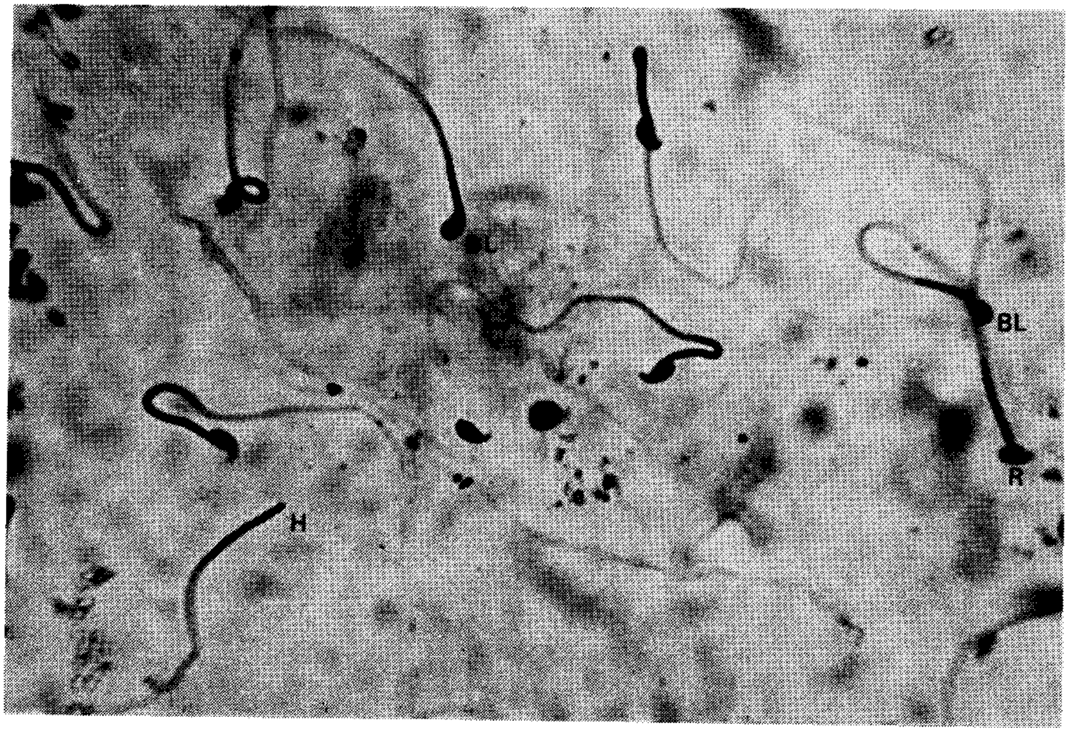

*Fig. 19. Sperms obtained from mouse administered orally with 460 mg/kg dose of Malathion. Notice the presence of balloon (**Bl**) and rhomboid (**R**) types of abnormalities as well as the headless sperm (**H**) as seen under the light microscope (X437).*

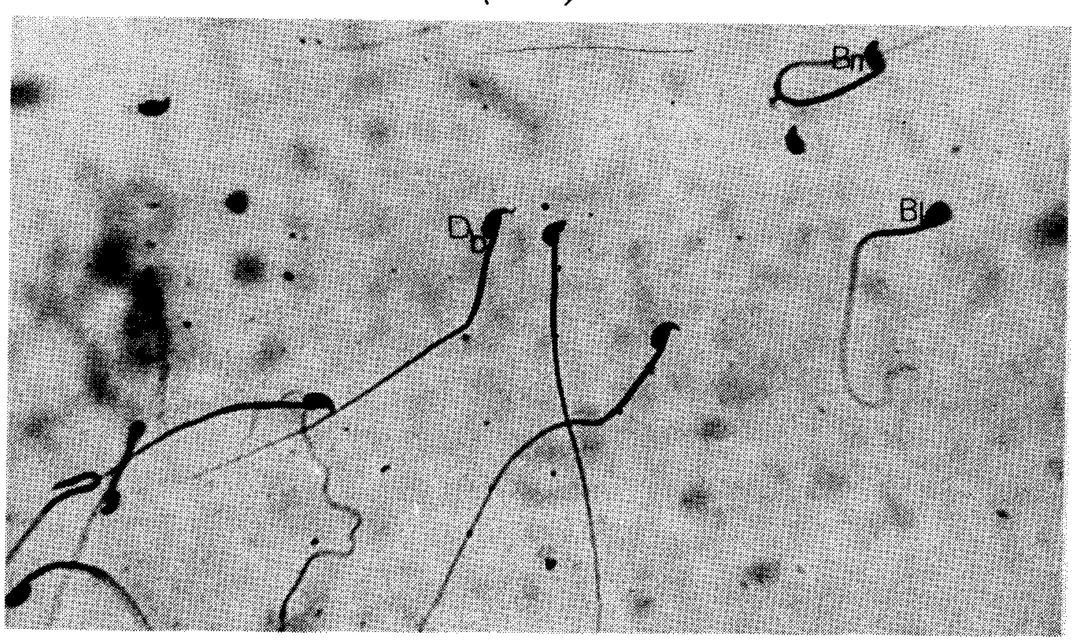

*Fig. 20. Sperms obtained from mouse treated orally with 460 mg/kg dose of Malathion. The abnormalities were: balloon (**Bl**), banana (**Bn**), and distally branched (**Db**) types as observed under the light microscope (X437).*

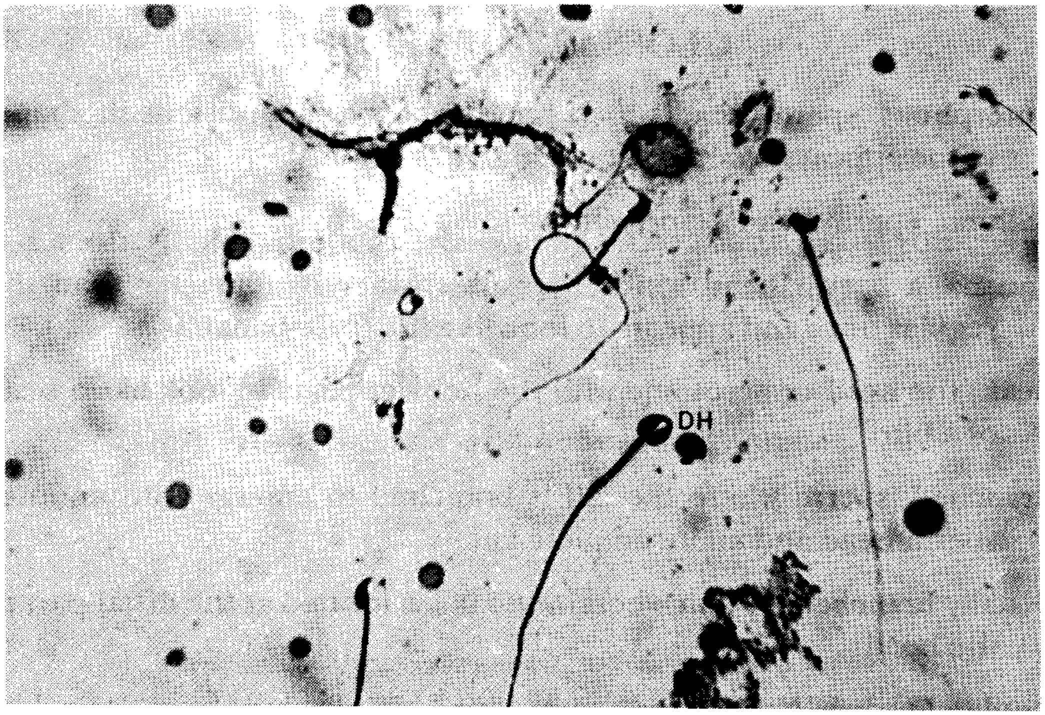

Fig. 21. Sperms obtained from mouse administered orally with 460 mg/kg dose of Malathion. Notice the double headed (**Dh**) *sperm abnormality as observed under the light microscope (X437).*

GLOSSARY OF TERMS

Amorphous sperm head which appears to be inconsistent in shape; vague appearance in cell membrane

Balloon sperm head shape with no hook present

Banana a sperm head which appears smaller in diameter than the regular ones and appears to look like the fruit, banana

Beak the head or acrosome with the hook appears to look like a beak of a bird

Branched sperm where the tail is branched to emerge with another sperm head and an incomplete tail

Distally branched sperm wherein the tail is located at the distal part of the head

Double headed two-headed sperm with equal distance from the tail

Funnel sperm head shape looks like an inverted triangle with no hook present

Rhomboid as the name implies there is the round shape of the head.

Sunflower sperm head shape which looks like a flower with petals where no definite cell membrane can be seen