120 hr. The 24 hr old eggs showed 40·0%, 22·0% and 4·0% hatching when refrigerated for 24, 48 and 72 hr respectively but no hatching was observed when they were refrigerated for 96 and 120 hr. Of the 48 hr old eggs 50·0%, 32·0%, 20·0% and 4·0% hatched after 24, 48, 72 and 96 hr of refrigeration but failed to hatch when refrigerated for 120 hr. The hatching of 72 hr old eggs was not inhibited even by the longest refrigeration period of 120 hr and similar was the case with 96 and 120 hr old eggs. In the controls maintained at 30° C the eggs showed a 100% hatchability.

These observations indicate that eggs in advanced stages of development are not so adversely affected by lower temperatures as the ones in early stages. The duration of the incubation period was found to be directly proportional to the refrigeration time. In 72 hr old eggs, it was 9 days when the eggs were refrigerated for 24 hr and 14 days when the refrigeration period was extended to 120 hr. Similarly in 96 and 120 hr old eggs the incubation period was extended with the extension of refrigeration period.

The authors are indebted to Dr. S. D. Loiwal, Principal of the College and Dr. O. P. Saxena, Head of the Department, for providing necessary research facilities. One of them (MG) is thankful to C.S.I.R. for the award of a Junior Research Fellowship.

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IMPACT OF GAMMA RADIATION ON THE MALE GERMINAL CELLS OF THE ERI SILKMOTH, *PHILOSAMIA RICINI* H. (SATURNIDAE : LEPIDOPTERA)

THE nature and frequency of chromosomal breaks induced by Co^{60} γ-radiation in the primary spermatocytes of *P. ricini* H. were studied. Irradiation by an acute dose of 1000 R of γ-rays in the prophase I and metaphase I cells resulted in chromatid and isochromatid types of breaks, the frequency of such chromosomal breaks being 8·4%. None of the subchromatid type aberrations were observed.

Introduction

Impact of ionising radiations like X-rays and γ-rays in inducing chromosomal aberrations has been elaborated in many species of plants and animals[1–6]. Basically three types of chromosomal aberrations have been stated to be produced. Induction of chromatid type break (involving one of the sister chromatids) and isochromatid type breaks (involving both the sister chromatids at the same locus) occur if cells are irradiated at the prophase or prometaphase (post-meiotic synthetic period of DNA)[7–8]. Subchromatid exchanges also are produced between chromosome units smaller than single chromatid during this period[9–10]. Chromosome type breaks are produced when the cells are subjected to irradiation at the resting stage (G_1 phase), prior to DNA synthesis[7–8]. However, both chromosome and chromatid type breaks are induced when irradiated during early S phase when there is incomplete replication of the chromosomes[11]. The purpose of the present investigation is to study the nature and frequency of occurrence of such stage specific induced chromosome breaks in the primary spermatocytes of the eri silkmoth, *Philosamia ricini* H.

Materials and Methods

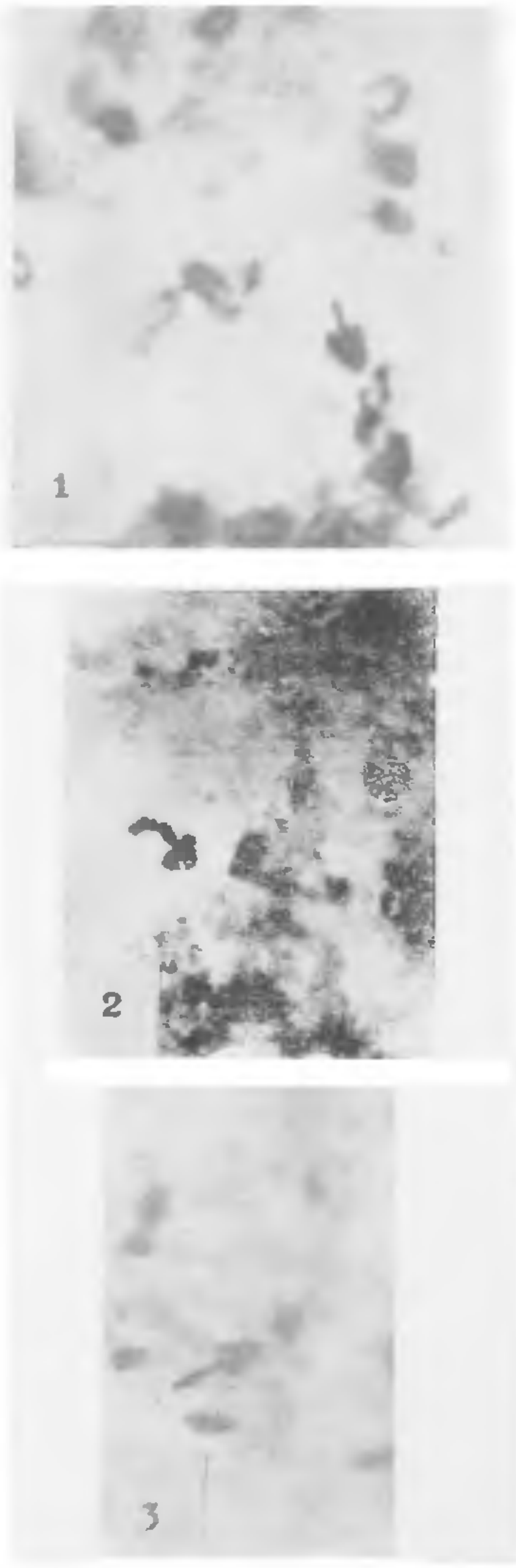
Male pupae of the species were irradiated during early half of their pupal life from a γ-ray source of Co^{60} (1·3 MeV, Eldorado–6) at a dose rate of 65R/min amounting to a total dose of 1000R, at the Cancer Institue, Cuttack. They were reared at a temperature of 28° C. The testes were excised after 8 hours and fixed in 3 : 1 ethanol acetic acid mixture. Chromosome preparations were stained by Heidenhein's iron haematoxylin.

Cytology of spermatogenesis of the species : The cytology of spermatogenesis in *P. ricini* follows a definite pattern. Gonial mitoses are quite abundant in the II instar larvae, just 7–8 days after hatching. The primary spermatocytes differentiate during the early III instar and are at the leptotene and zygotene stages. In the early IV instar, however, majority of the cells are in the pachytene stage. During the V_{3-4} instar the germ cells are in the diakinesis and within one day they reach the metaphase I. The duration from the very differentiation of an early primary spermatocyte to first meiotic metaphase is about 13 days. Early pupae show all stages including primary and secondary spermatocytes.

Results and Discussion

There is no marker chromosome, nor morphologically distinct sex chromosome in *P. ricini*. Two or more chromosomes are morphologically alike, either oval or spherical at metaphase I. Therefore, a study of the frequency of chromosomal breaks specific to marker chromosome or sex chromosome[12] is impeded. Further, it is not possible to distinguish chromosome or chromatid type breaks in the oval or spherical metaphase chromosome. Therefore, attempts, have been made to study them in the chromosomes during the diplotene and diakinesis at the time when the bivalents are elongate and pose the initiation and separation of their chiasmata. In these stages, displacement in the axes of the broken ends of a chromosome or chromatid is not expected. Chromosomal breaks have also been scored in meiotic metaphase cells although either

one-chromosome or chromatid type—could not be vindicated.



FIGS. 1–3. Figs. 1–2. Diakinesis cells of *P. riciri* showing isochromatid breaks (indicated by arrows), ×2,400. FIG. 3. Metaphase cell showing chromosomal break (indicated by arrow), × 1,600.

TABLE I

Frequency of breaks due to irradiation of 1000R of γ-rays after 8 hours in the male germinal cells of P. ricini *H*

Stages	No. of cells observed	Breaks: Subchromatid	Chromatid	Isochromatid	Chromosome
Diplotene	216	..	8	48	..
Diakinesis	148	..	..	20	..
Metaphase I	792	..	22	..	..
Pooled	1156	..	8	90	..
Control	1287	..	..	..	..

As shown in Table I, 8 are chromatid type and 48 are isochromatid type breaks in 216 diplotene cells and 20 are of the latter type in 148 diakinetis cells (Figs. 1 and 2). All the breaks in the metaphase cells are either chromatid or isochromatid types or a mixture of both types (Fig. 3). For analysis of breaks all the types (chromosome or chromatid) are added together and showed 8·4% breaks in 1,156 cells examined, 8 hours after treatment with 1000R of γ-rays. Out of these 2·2% were chromatid and 18·6% were isochromatid types in the prophase whereas not a single break (chromosome or chromatid type) was observed in 1,287 cells of control preparations.

Since, the time required for pachytene nuclei to reach the diplotene stage is about 7 days and to reach metaphase I, it is about 13 days, exposure to irradiation during these periods affects the corresponding prophase I and metaphase I chromosomes producing chromatid and isochromatid type of chromosomal breaks. None of these cells showed subchromatid exchange type breaks. Chromatid type breaks have been observed in metaphase I cells of *P. cynthia* irradiated during prophase I period (Nayak and Padhy, unpublished). Subchromatid type aberrations have not been observed in the irradiated meiotic cells of this species although reports on their occurrence have been made earlier[9,10].

The authors are thankful to the U.G.C. for rendering financial help for the present work. Thanks are also due to Major G. P. Mohanty, Principal, B.J.B. College and Prof. C. C. Das, Berhampur University, for encouragements. Finally we owe our gratitude to

Prof. N. Pradhan, Cancer Institute, Cuttack, for permitting the γ-ray source.

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SUPPRESSION OF MOUSE FERTILITY AFTER ADMINISTRATION OF DERIVATIVES OF CHALCONE

FAILURE of nidation has been described in female rodents mated with oestrogen-treated males; the effect conjecturally resulting from either transport of oestrogen in the semen to the vicinity of the uterus causing expulsion and degeneration of the fertilized eggs[1–3] or functional atrophy of the male sex structures[4]. This study is a preliminary attempt to evaluate the effect on fecundity of female mice mated with males treated with two promising non-steroidal derivatives of chalcone (benzalacetophenone), namely, 2′-chloro-3 : 4-methylenedioxy-4′-flourcchalcone (CH–6) and 2′-hydroxy-3 : 4 methylenedioxy-4′-flourochalcone (CH–8), reported earlier to possess oestrogenic and postcoital antiferitility activities[5].

The animals used were 2·5 to 3 months old adults (28–32 gm body weight) of proven fertility from the departmental colony of Swiss albino mice. The compound CH–6 or CH–8 was injected intraperitoneally to male mice in daily individual doses of 1·5 or 5 mg in 0·1 ml olive oil for 5, 10 or 15 days. Control males were similarly treated but with the oily vehicle alone. At least 5 mice from each group of the 1·5 mg dose treated animals were killed 24 hr after the last injection while the remainder were used for mating and testing of fertility. The testes, seminal vesicles and cauda epididymides of the sacrificed animals were dissected out, cleared from adherent tissue and weighed to the nearest mg on a torsion balance. For histological observations tissues were fixed in Bouin's fluid, sectioned and stained in Harris' haematoxylin and eosin. Males designated for mating were individually caged with a pro-oestrous parous female. Mating in each case was visually recorded and also confirmed by the presence of spermatozoa in the vagina or by a vaginal plug. The day of introduction of the male was taken as day 0. The mated females were autopsied on day 15 *postcoitum* and the number of uterine implantation sites, if any, were recorded. The results were statistically evaluated by the Student *t*-test.

Libido of the experimental males was not impaired after treatment with CH–6 or CH–8. Table I shows the antifertility activity of CH–6 and CH–8. A significant change in the rate of implantation in females mated with 1·5 mg/day CH–6 or CH–8 5-day treated males did not occur. However, total sterility was produced in females if matings were done with 10-day CH–6 or 15-day CH–8 treated males. At approximately the triple dose of 5 mg/day of CH–6 or CH–8 sterile matings resulted only after 5 days of treatment.

TABLE I

The effect on fertility of pro-oestrous female mice mated with CH–6 and CH–8 treated males

Male		Female	
Treatment	Days of injection	No. of mated	No. of implantation sites/mated female (Mean value ± S.E.M.)
None	..	6	8·1 ± 1·1
Vehicle alone	5–15	6	8·0 ± 0·9
CH–6 (mg/day)			
1·5	5	6	5·4 ± 0·8
1·5	10	4	0
1·5	15	5	0
5·0	5	5	0
CH–8 (mg/day)			
1·5	5	5	6·8 ± 1·0
1·5	10	4	4·4 ± 0·4
1·5	15	5	0
5·0	5	5	0

With a view to examining the mode of action of the chalcone derivatives, the effect of daily dose of 1·5 mg of CH–6 and CH–8 on the weight and histology of the testes, seminal vesicles and cauda epididymides was observed (Table II). A progressive decline in the weights of the reproductive organs occurred after 5, 10 and 15 days with either of the compounds. In