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THE EFFECT OF ETHYL METHANE SULFONATE (EMS), MALEIC HYDRAZIDE (MH) AND POTASSIUM CYNIDE (KNC) ON MEIOTIC CHROMOSOMES OF *ZONOCERUS VARIEGATUS* LINN.

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Abstract

EMS, MH and KCN were observed to induce abnormal choromosome behaviours and aberrations at various levels and also to impair meiotic processes, particularly choromosome pairing, in *Z. variegatus*. Aberrations observed include fragmentation, chromosome breaks, clumping, pulverization and nonpairing at pachynema, diplonema and diakinesis; clumping, nonpairing and multiple association at first metaphas, as well as lagging chromosomes, dicentric bridges and nondisjunction at anaphase I. The degree of impairment increased with the dose and duration of treatment. It was also noticed that in their action the mutagens have a delayed effect of about 24 hours.

Introduction

Zonocerus variegatus Linn. is an insect pest which is very abundant in many parts of Nigeria. It is commonly called the variegated grasshopper and belongs to the famuly of the locust.

The insect starts its life cycle as a little nymph which hatches from an egg and moults through five to seven instars to a green variegated adult (Youdeowei, 1974). Much work has already been done on the biology of the insect (Oyidi, 1968; Toye, 1971; Taylor, 1972; Lasebikan and Olorode 1972; Olorode and Akingbohungbe, 1975).

There has been some work also on the chromosome of the insect (Oyidi, 1967, 1968; Lasebikan and Olorode, 1972). The occurence of certain chromosomal aberrations in the natural populations of the insect was reported by Olorode and Akingbohungbe (1975).

The male insect which forms the material for this work has nineteen chromosomes made up of nine pairs of autosomes and one heteropycnotic X-chromosome, (Oyidi, 1967, 1968). Lasebikan and Olorode (1972) formally described the karyotype of this insect.

Much has been done on artificially induced mutations and chromosoma aberrations in both plants and animals using chemical mutagens as well a ionizing radiations. However, most of the publications have been based o

effects of mutagens on mitotic chromosomes (Kihlman, 1950; Huges and Spraggs, 1958; Evans and Scott, 1964; Scalera and Ward, 1971; Kelly and Legator, 1971).

EMS, MH and KCN are standard mutagens and have been shown to induce mutations and aberrations in stored cells, that is sperms and seeds and their mechanism of action have also been reported (Evans and Scott, 1964; Scalera and Ward, 1971). It could be expected therefore that such mutagenic effects as would be reported for meiotic cells of *Z. variegatus* are due to the same mechanism. So far there has not been any report on mutagenic effects on meiotic cells of *Z. variegatus* possibly because at present very little has actually been done on effect of mutagens on meiotic processes.

EMS is an alkylating agent with a functional ethyl group. It is an oily fluid and only partially soluble in water. MH is a structural isomer of the nuclear base of RNA — Uracil. It is an amorphous powder that is fairly soluble in water. KCN is a well known metabolic poison. It is crystalline in form and well soluble in water.

A preliminary study by Olorode and Akingbohungbe (unpublished) has shown that EMS and MH will induce aberrations in the meiotic chromosomes of *Z. variegatus*. The present study was conducted to investigate the mutagenic effect of EMS, MH and KNC on meiotic chromosomes of *Z. variegatus*. The chemicals were applied *in vivo* and their effects cytologically analysed. It is expected *a priori* that the mutagens will impair meiotic processes and produce morphological defects on chromosomes of Z. variegatus. Such effects are expected to lead to sterility.

This study is a preliminary attempt to monitor the prospects of chemosterilants in the genetic control of *Zonocerus variegatus*.

Materials and Methods

A large population of male *Z. variegatus* totalling 600, in the fifth and sixth instars was collected from the field around the University of Ife Teaching and Research Farm between February and March. This corresponds to the dry season population as described by Youdeowei (1974). These were held in ten wood-framed wire gauze cages 25 cm x 25 cm x 40 cm and kept in the laboratory at a temperature of 25 $\pm$ 2°C. The nymphs were fed on pawpaw (*Carica papaya* L) leaves until they moulted into adults.

The adults were collected as they emerged and grouped into age classes of five day-day intervals. The adults were held in white transparent plastic containers, 12 cm in diameter at the top and 15 cm high, tapering slightly towards the bottom. The top of the containers was closed with wire-gauze.

The mutagens EMS in 10-gram bottles and MH powder were obtained from Koch-Light Laboratories Limited, England and 97% pure crystalline KCN from BDH chemicals Limited, Poole England. EMS was measured in millilitres while MH and KCN were measured in milligrams.

The following concentrations were used: 2 units for 5 days, 2 units for 2 days, 2 units for 1 day, 1 unit for 5 days, 1 unit for 2 days and 1 unit for 1 day.

Owing to the higher toxic effect of the mutagens, determined by the decrease in survival of the insects, concentrations higher than 2 units for 5 days were discarded.

The mutagens were introduced straight into 10 grammes of Drosophila food used as the treatment medium and fiv insects were treated in each regime.

Testes were extracted from the insects and fixed in acetic alcohol (acetic acid and 95% ethanol in a 1:3 ratio), and stored in labelled vials. All the vials, each containing testes from single insects, were kept in a regrigerator until examined.

Cytological examinations were conducted using a modified orcein squash technique (Olorode, 1974). The various aberrations and frequency of occurrence were scored against each treatment.

Photomicrographs were taken from good preparations. The frequency of each aberration was scored as a percentage of the total number of cells observed at each stage of division.

Result and Observations

These mutagens-induce aberations at all stages of division possibly because they do not inhibit division but attack the chromosome structure as well as distort meiotic processes. This means that aberrations induced early in division had a chance of being expressed in subsequent stages of division. The frequency of occurrence and type of aberrations are represented in Tables I–IV. In all cases the greatest effects were observed in 2-units-for-5-days and 1-unit-for-5-days regimes.

Pachynema and Diplonema

At pachynema and diplonema, the aberrations consist mainly of fragmentation and nonpairing (Table I; Figure 1 and 2). Fragmentations were generally greater with KCN and slightly fewer with EMS. Nonpairing was greater with EMS. It is possible that other types of aberrations were present at these stages but not detectable.

Diakinesis and Metaphase I

At diakinesis and metaphase I, many cells were observed in which the chromosomes are bunched together instead of occurring as distinct bivalents. This is referred to as clumping (Fig. 3). The occurrence of certain chromosomes as univalents at diakinesis, a situation termed nonpairing was also observed (Fig.4). The frequencies of diakinesis and metaphase-I aberations

TABLE I

EFFECT OF MUTAGENS ON PAGRYNEMA AND DIPLONEMA

TREAMENT	PACHYNEMA							DIPLONEMA						
	NONPAIRING			FRAGMENTATION			TOTAL[a]	NONPAIRING			CHROMOSOME BREAN			TOTAL
	EMS	MH	KCN	EMS	MH	KCN		EMS	MH	KCN	EMS	MH	KCN	
2/5	24.5	26.4	0.0	64.4	61.5	99.3	209	4.9	62.8	16.7	7.5	11.6	56.6	89
2/2	25.3	9.3	19.1	50.0	53.9	66.2	212	50.0	54.2	68.3	9.1	8.3	21.0	201
2/1	18.4	8.6	0.9	14.0	17.3	3.4	190	25.5	19.5	1.3	4.3	0.0	1.4	173
1/5	3.9	12.8	0.0	54.4	62.8	88.7	192	36.1	36.8	36.8	4.9	10.3	31.9	195
1/2	18.7	19.7	16.7	27.6	37.9	55.1	150	36.7	39.1	20.0	2.2	4.4	2.3	131
1/1	0.0	1.0	0.9	2.0	4.9	6.9	162	6.8	9.8	0.0	0.0	0.0	1.1	112
Control	0.0	0.0	0.0	0.0	0.0	0.0	90	0.0	0.0	0.0	0.0	0.0	0.0	69

Values in this Table (and susequent Tables) represent the frequency of cells carrying aberrations expressed as a percentage of total number of cells observed per stage of division in each treatment regime.

a. This value represents the total number of cells observed.

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TABLE II

EFFECT OF MUTAGENE ON DIAKIMENSIS AND METAPHASE I

TREATMENT	DIAKINESIS										RETAPHASE I									
	NONPAIRING			CLUMPING			MULTIPLE ASSOC.			TOTAL	NONPAIRING			CLUMPING			MULTIPLE ASSOC.			TOTAL
	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN		EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	
2/5	49.0	6.6	1.4	23.9	14.4	77.0	18.1	60.1	20.3	122	38.9	42..2	35.2	54.9	55.0	59 0	2.0	0.0	7.7	81
2/2	38.1	41.0	31.8	40.5	26.2	25.8	4.9	17.4	19.7	91	49.1	47.3	48.5	46.6	43.9	42.4	0.6	0.0	0.0	95
2/1	0.0	1.4	4.9	14.6	7.0	6.2	17.2	25.4	0.0	85	2.9	0.0	0.0	18.7	24.2	7.1	0.0	6.3	0.0	107
1/5	87.1	44.4	34.8	5.5	9.9	19.6	4.1	25.9	23.9	108	25.5	50.7	44.8	65.7	58.4	49.9	0.0	2.3	1.0	217
1/2	8.0	0.0	0.0	19.0	3.9	10.3	20.8	19.4	9.9	76	24.5	4.0	2.6	48.9	32.0	20.6	0.0	10.0	0.0	79
1/1	4.3	6.6	0.0	2.9	0.0	0.0	11.4	0.0	0.0	43	10.0	0.0	6.3	0.0	0.0	0.0	0.0	2.5	0.0	116
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	79	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	83

are generally slightly higher in the EMS treatments, (Table II). Since nonpairing was also observed at earlier stages of division (at pachynema, for instance), its occurrence at later stages seems to indicate failure of synapsis rather than precocious separation. The unpaired homologues seem to exhibit delayed congression at the metaphase plate and they consequently lag at anaphase I.

Anaphase I and Telophase I

At anaphase I interesting defects become evident: these include lagging chromosomes (Fig. 5.), dicentric bridges, nondisjunction (Fig. 6). fragmentation and clumping. The occurrence of unsynchronised anaphase movement was very low.

Lagging chromosomes refer to chromosomes that remain at the metaphse plate while the others migrate to the poles. In most cases of lagging recored, the smallest and medium-sized chromosomes or bivalents were involved. A similar observation was made for nonpairing chromosomes. A cursory examination of the size of the lagging chromosomes indicates that they can be resolved almost invariably into pairs of homologues and if there are four they are two pairs of homologues.

In most cases of lagging, the laggards underwent equational division (Fig. 5). There is no certainty as to what ultimately happened to the lagging chromosomes. It is probable that in a few cases the laggards simply get lost. The values for frequencies of aberrations at anaphase I and telophase I are present in Table III.

The X-chromosomes generally migrate in advance of the autosomes. This is possibly due to the fact that there is a precocious replication and subsequent condensation of the X-chromosomes (Grumbach, *et al*, 1963). Where the X-chromosomes formed an association with an autosome, usually one of the unpaired small chromosomes, the anaphase movement of the X-chromosomes is syncronised with that of the autosomes.

Aberrations such as nondisjunction and unsynchronised migration were reported by Olorode and Akingbohungbe (1975).

Metaphase, anaphase and telophase of second division

Most of the severe aberrations persisted through first division into second division. One implication of this phenomenon is that the chemicals persisted in their action throughout the period of treatment and thus blocked any mechanism that would otherwise have caused a repair of such defects (e.g. the proper rejoining of a breakage).

Pulverization, clumping and lagging were fairly frequent during second division while bridges and fragmentation were rare. These results are summarised in Tables IV, V and VI.

TABLE III

EFFECT OF MUTAGENS ON ANAPHASE I

TREATMENT	ANAPHASE I																		
	NONDISJUNCTION			DICENTRIC BRIDGES			FRAGMENTATION			LAGGARDS			UNEQUAL MIGRATION			CLUMPING			TOTAL
	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	
2/5	13.6	11.3	12.1	9.5	2.6	10.5	5.5	3,9	23.7	27.2	57.6	10.6	1.5	2.6	9.5	29.0	16.9	31.6	127
2/2	14.7	10.8	13.3	8.8	5.1	9.6	7.5	6.3	21.7	36.2	52.1	13.2	0.0	6.3	6.0	15.7	21.3	10.5	184
2/1	1.1	1.2	6.2	0.0	0.0	0.0	1.2	0.0	0.0	6.5	6.7	6.2	9.0	0.0	0.0	0.0	7.3	3.7	96
1/5	16.3	19.1	12.5	7.1	23.5	14.1	9.6	23.6	21.9	45.6	20.0	21.8	2.0	0.0	0.0	15.2	12.2	23.4	118
1/2	11.2	11.1	8.1	0.0	7.3	10.8	0.0	17.3	12.2	33.3	37.1	28.0	6.1	4.9	0.0	12.6	15.7	13.5	60
1/1	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	98
Control	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	102

TABLE IV

EFFECT OF METAGENS ON TELOPHASE I

TREATMENT	TELOPHASE I									
	CLUMPING			DICENTRIC BRIDGES			LAGGARDS			TOTAL
	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	
2/5	40.0	43.4	33.7	20.0	21.2	9.7	32.0	26.3	45.1	203
2/2	29.1	24.7	13.0	18.3	12.4	8,7	27.6	45.4	63.1	187
2/1	4.2	7.8	5.6	0.0	3.1	0.0	5.9	6.3	4.5	94
1/5	51.4	34.2	23.5	12.5	13.2	9.2	26.8	44.7	53.0	104
1/2	8.1	15.7	17.9	10.9	11.9	10.3	21.2	50.7	23.1	85
1/1	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.3	0.0	83
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	87

TABLE V

EFFECT OF MUTAGENS ON THE SECOND MEIOTIC DIVISION

TREATMENT	METAPHASE II				ANAPHASE II												
	CLUMPING			TOTAL	DICENTRIC BRIDGES			FRAGMENTATION			LAGGARDS			UNEQUAL MIGRATION			TOTAL
	EMS	MH	KCN		EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	EMS	MH	KCN	
2/5	96.8	92.6	89.4	75	24.8	25.0	22.1	0.0	12.3	18.2	26.1	24.5	15.6	31.5	34.2	31.2	89
2/2	59.9	84.2	78.6	91	0.0	6.7	6.8	11.2	0.0	28.8	29.1	31.5	10.8	7.0	17.9	9.6	97
2/1	7.9	4.1	6.8	86	0.0	0.0	0.0	0.0	0.0	1.2	4.7	4.9	3.7	0.0	1.0	0.0	135
1/5	94.1	93.2	88.4	88	11.7	5.8	6.7	1.8	7.2	11.1	28.5	50.1	45.5	28.5	30.4	18.9	153
1/2	39.0	88.5	61.5	95	0.0	0.0	0.0	3.3	17.6	12.9	10.3	21.2	32.3	14.7	14.1	0.0	150
1/1	0.0	7.2	0.0	63	0.0	0.0	0.0	0.0	0.0	0.0	2.6	1.3	0.0	0.0	5.2	0.0	93
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	87

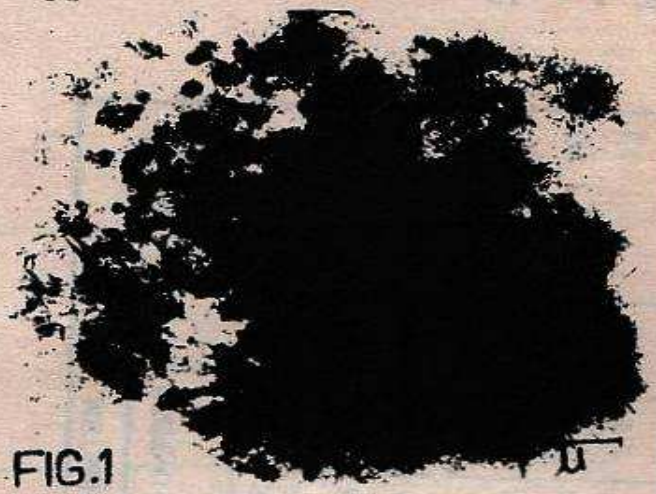

Fig. 1: Profuse fragmentation at pachynema induced by EMS 2 ml for 5 days.

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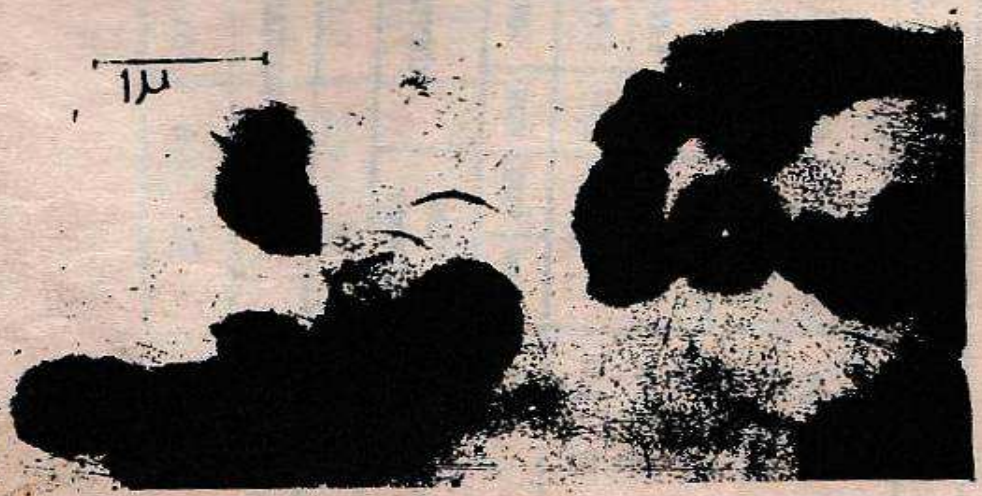

Fig. 3: Clumping at diakinesis (two cells) induced by KCN 2 ml for 2 days. Arrow indicates heterochromatin connection between the X- chromosome and one autosome.

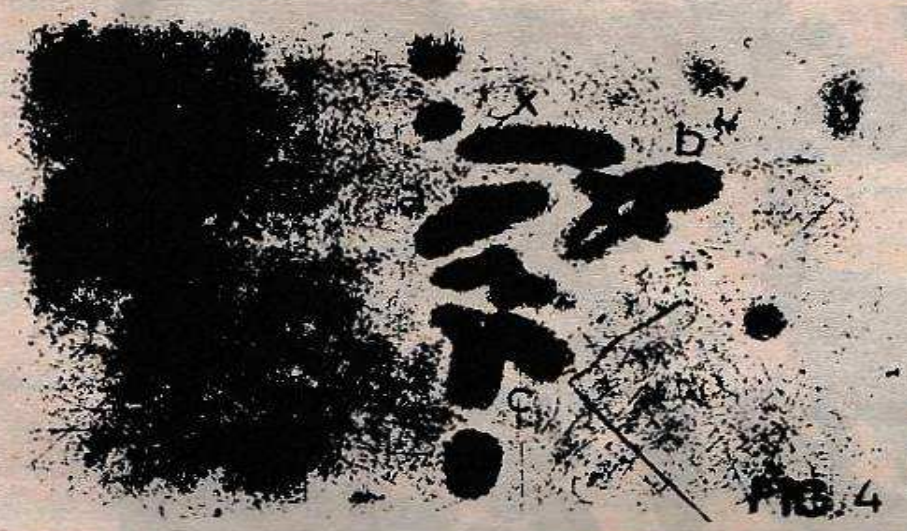

Fig. 4: Nonpairing at diakinesis induced by EMS 2 ml for 2 days. Only three homogous pairs (a, b, and c) are completely paired. X denotes the X- chromosome.

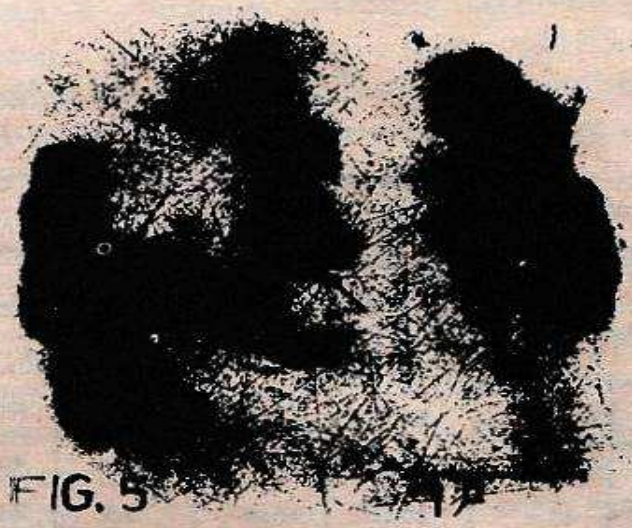

Fig. 5: Lagging at first anaphase induced by EMS 2 ml for 5 days. Arrow indicates one of the anakkest cgrinisines with evidence of equational division.

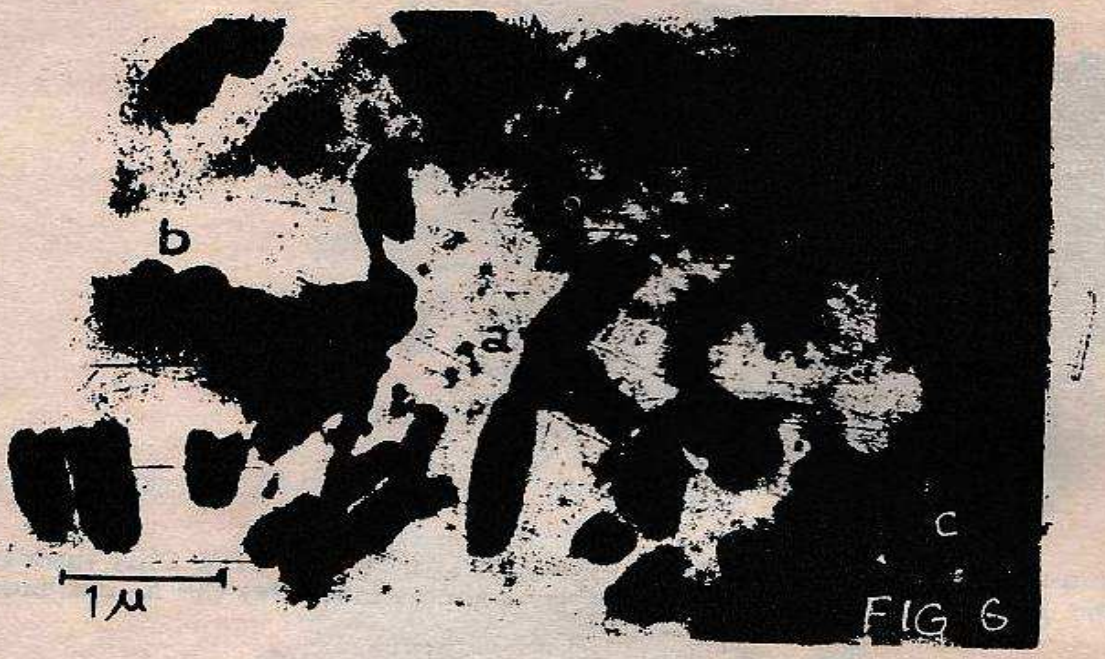

Fig. 6: Multiple aberrations at first anaphase induced by MH 2 mg for 5 days. a denotes nondisjunction, b denotes lagging and c indicates fragmentation.

TABLE VI

EFFECT OF MUTAGENS OF SECOND TELOPHASE

TREATMENT REGIME	CLUMPING			LAGGING			TOTAL
	EMS	MH	KCN	EMS	MH	KCN	
2/5	42.9	56.3	41.4	30.4	6.2	40.2	117
2/2	35.2	47.8	32.6	16.3	9.9	23.6	227
2/1	0.8	4.0	4.3	1.1	0.0	2.9	125
1/5	50.1	80.5	39.7	19.6	11.5	15.1	162
1/2	21.7	40.3	20.3	9.0	8.2	13.6	183
1/1	0.0	3.2	0.0	0.2	0.0	0.0	89
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	99

Discussion and Conclusion

The reactions of various mutagens have been studied at various levels of complexity, using biological materials ranging from lower to higher plants and animals as well as cultured cells of human tissue. The modes of action of mutagens have also been discussed. But from this work there seems to be no single overall mechanism or cause which can explain all the aberrations since they may be induced through different mechanisms. The mechanisms discussed below do not preclude other indirect modes of action yet undiscovered.

Mechanism of action of EMS

It has been suggested that the alkylating agents act by attacking DNA and even RNA at the N-7-atom moieties (Brook and Laowley, 1961). The alkylation at the 7-N-position of guanine uses the free electron pair of the imidazole ring to bind the ethyl group causing full possitive charge. This possitive charge distributed to both N atoms of the imidazole ring greatly weakens both the 7-N-ethyl and 9-N-sugar bonds so that hydrolysis can occur at room temperature and natural pH (Bantz and Freese, 1960). The reaction creates lesions in DNA backbone and these lesions can result in such aberrations as chormosome breakage and fragmentation.

The anaphase I dicentric bridges reported could have resulted from chromatid breaks close enough in space ant time to allow reunion of non-sister chromatids. At anaphase, homologous chromosomes move to opposite poles directed by their centromeres. As the contromeres associated with such non-sister chromatids move to opposite poles, a bridge/bridges is/are formed. Since these mutagens induced fragmentation and chromosome breaks, it is not unexpected that such bridges would result. The ultimate fate of the bridges is not entirely known.

Alkylating agents such as EMS produce linkage of two guanine moieties by alkyl chains (Brook and Lawley, 1961). If such multiple linkage may lead to clumping ot the chromosomes, the intensity of clumping will depend on amount of mutagen as well as the amount of guanine sites available.

The alkylation of 7-N-atom also creates a positive electrical charge. If such electrical charges are created sufficiently all along the chromosomes it is possible that nonpairing of homologous chromosomes may result primarily from the repulsion of like charges (Rapp, *et al*, 1977).

Mechanism of action of MH and KCN.

Except for minor differences, the aberrations produced by MH and KCN are similar to those produced by EMS. The trend is also the same; i.e. greater effect at higher concentrations and longer durations of exposure. However, the modes of action are believed to be different.

MH is known to attack the SH group in proteins including nucleo-proteins and involved in DNA metabolism (Huges and Spraggs, 1958). Such a reaction may distort chromosome behaviour and thus lead to discrepant morphology.

KCN, a metabolic poison, inhibits cellular activities: this means that the overall effect on mitotic and meiotic processes would be similar to that of other chemicals reported above.

Generally, all the aberrations observed could be modifications of a single effect or single action exerted at different times, or one aberration could be a consequence of another. For example breakage and fragmentation could result in bridges and clumping while nonpairing could result in lagging etc. Whatever the case, the results obtained suggest an impairment of important cellular functions which are related to mitotic and meiotic events.

It seems conclusive from the data therefore that the mutagens EMS, MH and KCN induce various aberrations *in vivo* in meiotic cells of *Z. variegatus* and that the degree of induction of such aberrations is dependent on dose level and duration of exposure.

It seems obvious that the chromosomal aberrations observed in this study are likely to produce non-viable gametes which means sterility. Similarly, various genetical and cytological modifications may arise in the subsequent generations of treated animals.

Chromosome-based sterility techniques have obvious potentials in genetic control of insect populations (Wagoner *et al.*, 1974). Similarly, chromosome aberrations that survive into advanced generations may be screened to produce aberrant homozygous stocks which can be mass-reared and released to produce sterile field hybrids and thus suppress field populations (Pal and Whitten, 1974).

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