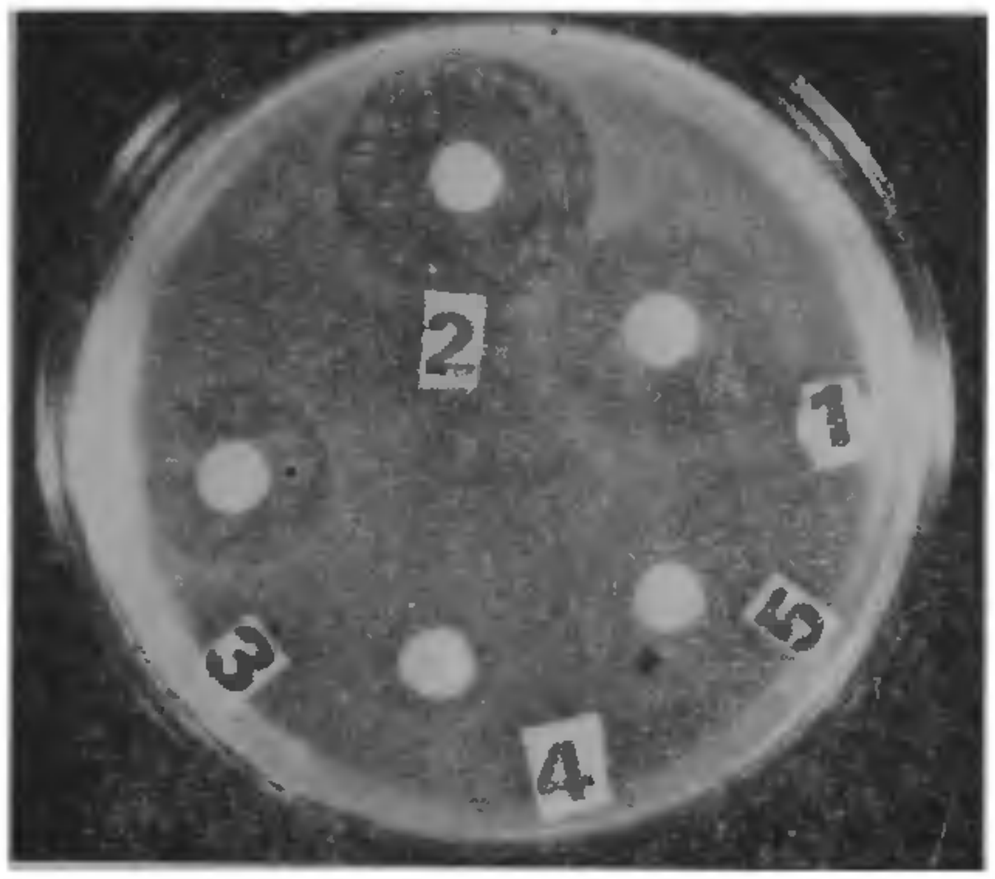

FIG. 1. Zones of inhibition effected by mercuric sulfadiazine against *Ps. aeruginosa.*

1. Sulfadiazine 250 mcg (20 mm); 2. Mercuric sulfadiazine 111 mcg (25 mm); 3. Mercuric sulfadiazine 11 mcg (18 mm); 4. Mercuric sulfadiazine 1·1 mcg (12 mm); 5. Solvent (dimethylformamide).

of this increased activity of mercuric sulfadiazine compared, the compound deserves clinical studies in the treatment of burns and other *Pseudomonas* infections. The official yellow mercuric oxide eye ointment[8] uses a 1% ointment giving a 0·046 molar concentration of mercury which is almost ten times the concentration found in the mercuric sulfadiazine cream tried by us. Hence further experimentation in its clinical applicability is warranted. Nickel sulfadiazine also shows promising activity and may be further investigated.

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1. Fox, C. L. (Jr.), *Arch. Surg.*, 1968, 96, 184.
2. —, *Int. Surg.*, 1975, 60, 275.
3. *Extra Pharmacopoeia* (Martindale), 27th Ed., 1977, p. 1471, Pharmaceutical Press (London) (and references therein).
4. (*a*) Fox, C. L. (Jr.), Monafo, W. W. (Jr.), Ayvarzian, V. H., Skinner, A. M., Modak, S., Stanford, J. and Condict, R. N., *Surg. Gynaecol. Obstet.*, 1977, 144, 668.
   (*b*) Fox, C. L., Modak, S. and Stanford, J. W., *Ibid.*, 1976, 42, 553.
5. Nagesha, C. N. and Karuna Sagar, I., *Arch. Surg.*, 1978, 109 (in press).
6. Jain, N. K. and Kamal K. Chaturvedi, *Hindustan Antibiotics Bulletin*, 1975, 18, 40.
7. Chaturvedi, K. K., Agarwal, B. K., Siddiqui, S and Kaushal, R., *Indian J. Pharm.*, 1975, 37, 85.
8. *Indian Pharmacopoeia*, 2nd Edition, 1966, p. 444.

INTERPHASE SENSITIVITY AND SPECIFICITY OF SILIQUA MUTATIONS INDUCED BY E M S IN INDIAN MUSTARD

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ABSTRACT

Synchronised seeds of Indian mustard were treated with the mutagen E M S during G_1, earlier half S, later half S and G_2 phase of the mitotic cell cycle. Observations were recorded in M_2 generation on four different types of siliqua mutations. Mutation frequency on family basis was found to be higher when the mutagen was applied at S or G_2 as compared to G_1. While appressed pod mutants were observed in all the treatments, the other three types, *viz.*, curved pod, incurved pod and thickened pod mutants showed some specificity of mutagenesis with the mitotic cycle.

INTRODUCTION

CHANGING the spectrum of mutations in a predictable manner and thereby achieving directed mutagenesis is an important goal of current mutation research. Nilan[1] has reviewed the various reports of alteration in spectrum induced by specific mutagens and treatment conditions and has concluded that different mutagens and treatment procedures may induce some changes in the relative proportions of different types of mutations in higher plants. Differences in sensitivities to radiation and chemical induced

genetic changes have been of theoretical importance from the beginning and further exploration of these differences are of current practical importance. It is evident from the experiments on barley[2–7], on rice[8,9] that when seeds are treated with chemical mutagens such as E M S, Ethyline imine, DES or 5 BUdR during the period of DNA synthesis (S phase) of cell cycle, the mutation frequency is significantly higher. Further the studies of Cerda'-Olmedo *et al*[10] have shown conclusively in *E. coli* that maximum frequency of a given type of mutation occurs, when the treatment is given at the time of DNA replication. Data on higher plants suggest that the yields of gene mutations[9,11] and chromosomal aberrations[11,12] vary with the stage in the division cycle. The present investigation was undertaken to pursue the above findings in relation to viable mutations with particular emphasis on mutagen sensitivity during different component phases of interphase of the cell cycle and the specificity of mutagenesis in the different phases.

Experimental

Dry seeds of a commercial inbred of *Brassica juncea* (L.) Coss and Czern, var. T–59 were presoaked in water for 13 h and then immersed in aqueous solution of hydroxyurea (0·05%) for 15 h to synchronise the cells in the seeds [13 h presoaking of seeds results in the initiation of S phase of cell cycle and further 15 h treatment of hydroxyyurea (HU) of the said concentration arrests about 80% cells in the seed at G_1/S boundary without any lethal effect[13]]. Synchronised mustard seeds were treated with 0·6% aqueous solution of E M S for 3 h at different times after release from HU inhibition and thorough washing. Mutagenic treatments were administered at G_1, S_e, S_l and G_2 of the cell cycle by adjusting with the lapse of time from HU release. Mitotic cell cycle in Indian mustard var. T–59 is completed approximately within 12 h out of which G_1 lasts about 2·7 h, S 5·5 h, G_2 3·5 h[14]. DNA synthetic phase (S phase) has been further arbitrarily subdivided in the present study into earlier half (S_e) and later half (S_l) of three hours' each with 15 minutes common to both the sub-divisions of the S phase. For treatment at G_1 stage, the dry seeds were presoaked for 10 h then and treated witht he mutagen. Mutagenic treatment for G_2 cells was employed 6 h after HU release. Mutagen treated seeds were thoroughly washed for one hour and sown in seed beds. Each M_1 plant was selfed and the seeds were collected from the main fruiting axis. The M_2 generation was raised as plant to row progeny and the population was scored carefully for morphological mutations for shape of the fruits and their attachment to the peduncle. All M_1 treatments were given at 25 ± 2° C.

Results

The following four types of siliqua mutations quite different from each other were recorded besides many other morphological mutations in the M_2 families.

(*a*) Appressed pod mutant, where the siliquae are appressed to the fruiting axis,

(*b*) Curved pod mutant, where the siliquae are curved outward,

(*c*) Incurved pod mutant, where the fruit is short, thicker and little incurved towards the fruiting axis,

(*d*) Thickened pod mutant, with fruits thickened and short, clustering at the apex of the fruiting axis.

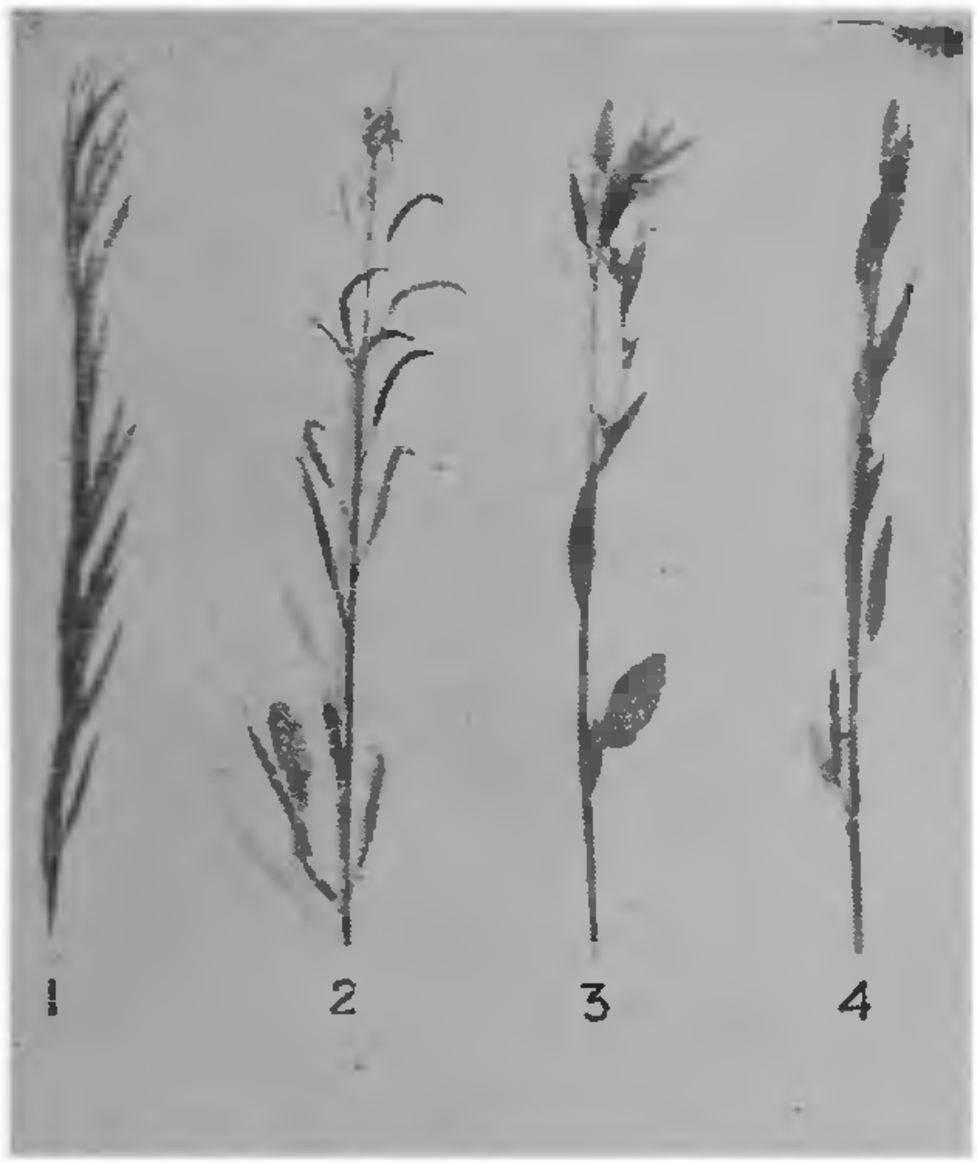
TABLE I*

Frequency of siliqua mutations in M_2 generation induced by E M S during different component phases of interphase

Component phase of Interphase		Total M_2 families studied	M_2 families segregating for siliqua mutations	Type of mutation induced (No. of M_2 families segregating for siliqua mutations)			
				Appressed pod	Curved pod	Incurved pod	Thickened pod
G_1 (presynthetic gap)		120	20	4	16	..	..
DNA synthetic phase	S_e (earlier half S)	116	56	4	52	..	..
	S_l (later half S)	112	48	4	8	36	..
G_2 (Post-synthetic gap)		120	56	12	..	28	16

* Similar experiment was also performed on one more variety (var. T–16) which reflected the similar results, but the data has not been included in the table, because of the fact that this M_2 population was severely affected by white rust causing malformation in some plant parts and in turn slightly hampering the true analysis.

Data are recorded in Table I. All the above mentioned siliqua mutations (Figs. 1–4) are reproduced in next generation.



FIGS. 1–4. Types of siliqua mutations. 1. Appressed pod, 2. Curved pod, 3. Thickened pod, 4. Incurved pod.

DISCUSSION

Onset of S phase in dry seeds of Indian mustard var. T–59 takes place after the seeds have been presoaked in aerated water for 13 h at 25° C as evidenced by ^{3}H-Thymidine incorporation[13]. Seeds, the favoured material for plant mutation studies, possess non-synchronised population of cells. Best possible effort has been made to synchronise the cells in the seed using HU for proper administration of mutagenic treatment on particular stage of the cell cycle. HU has a selective inhibitory effect only against DNA synthesis, particularly at lower concentrations. However, at higher concentrations it may affect RNA and/or protein synthesis and also can induce chromosomal aberrations[15]. Since the action of HU is readily reversible and since it inhibits mitosis mainly by inhibition of DNA synthesis in the S phase, it can be used as a cell synchronising agent. In the present study it has been found that when dry seeds presoaked in water for 13 h (*i.e.*, the time required for onset of S phase the knowledge of which is helpful in avoiding the unnecessarily prolonged HU treatment thereby minimising its lethal effect otherwise if any) are treated with aqueous solution of HU (0·05%) for 15 h, 80% of cells in the seed are arrested at G_1/S boundary which on removing the HU inhibition progress further synchronously to S and subsequent stages as evinced by ^{3}H-Thymidine autoradiography. Furthermore, the above dose of HU which is effective in inducing high degree of cell synchrony in this system does not affect seed germination, mitotic division (does not induce chromosomal aberrations), meiotic behaviour, pollen fertility and also does not induce visible mutations when HU treatment is employed to seeds[13].

It is clear from the observations documented in Table I that the mutation frequency is significantly increased when the treatment is administered during DNA synthetic phase or G_2 as compared to G_1. The enhanced frequency of viable morphological mutations in question, during S phase, may be because of the higher mutagenic susceptibility and possible S phase specificity. Similar results have been reported by earlier workers in higher plants particularly with chlorophyll deficient mutations[1, 8, 16]. A high frequency of induced mutations in G_2 may be due to specific treatment effect depending on the physiological state of the cell during treatment, *i.e.*, repair or enzyme function as reported by Auerbach and Ramsay[17]. Though every care has been taken for proper administration of mutagenic treatment at particular stage of interphase, it is possible that during G_2 treatment few of cells in the seed may be in late S phase and the high incidence of mutations observed in the G_2 population may partially be accounted to these S phase cells.

It is further evinced in the table that relative frequency of particular type of mutation is more in particular treatment. Appressed pod mutants are common in all the treatments but the other three types of mutations show some specificity of mutagenesis. Curved pod mutants are most frequently observed in S_e and incurved pod mutants in S_l. This may be assigned to their time dependent specificity during the DNA synthetic phase. Swaminathan and Sarma[6], Sarma[18] and Yamaguchi and Matsubayashi[3], Narahari[9] also observed some time dependent specificity of chlorophyll deficient and other viable mutations during DNA synthetic phase in barley and rice respectively. In G_2 treatment besides incurved pod mutants, some thickened pod mutants were also observed which may be attributed to the possible role of repair mechanisms or enzyme functions operating there. The results could also be explained by the differential effect of the mutagen on active versus inactive genes, the number and time of which vary during the cell cycle[19]

It is of interest from the present study that some specificity of a given type of mutation can be achieved through this approach which may be utilised in the screening of beneficial mutations.

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1. Nilan, R. A, *Proc. Symposium on Induced Mutations and Plant Improvements*, IAEA, 1975, p. 141.
2. Garina, K. P., *Izv. Akad. Nauk. SSSR. Ser-Biol.*, 1974, **1**, 95.
3. Mikaelser, K., Ahnström, G. and Li, W. C., *Hereditas*, 1968, **59**, 353.
4. Natarajan, A. T. and Shivashankar, G., *Z. Vererbungsl.*, 1965, **96**, 13.
5. Savin, V. N., Swaminathan, M. S. and Sharma, B., *Mutat. Res.*, 1968, **6**, 101.
6. Swaminathan, M. S. and Sarma, N. P., *Curr. Sci.*, 1968, **37**, 685.
7. Yamaguchi, H., *Env. and Exp. Botany*, 1976, **16**, 145.
8. —and Matsubayashi, I., *Mutat. Res.*, 1972, **17**, 191.
9. Narahari, P., *Indian J. Exp. Biol.*, 1978, **16**, 139.
10. Cerdá-Olmedo, E., Hanawalt, P. C. and Guerola, N., *J. Mol. Biol.*, 1968, **33**, 705.
11. Garina, K. P., *Proc. Ind. Natl. Sci. Acad. (Part B)*, 1975, **41**, 248.
12. Grant, C. J., Heslot, H. and Ferrary, R., *Chromosomes Today*, 1969, **2**, 75.
13. Lavania, U. C., *Sci. and Cult.*, 1977 *a*, **43**, 445.
14. —, *Ibid.*, 1977 *b*, **43**, 176.
15. Timson, J., *Mutat. Res.*, 1975, **32**, 115.
16. Swaminathan, M. S., *Proc. Symposium on Induced Mutations in Plants*, IAEA, 1969 p. 719.
17. Auerbach, C. and Ramsay, D., *Molec. Gen Genet.*, 1968, **103**, 72.
18. Sarma, N. P., "Studies on the planned alteration of the spectrum of induced mutations in barley," *Ph.D. Thesis*, IARI, New Delhi, 1971.
19. Brock, R. D., *Proc. Symposium on Induced Mutations in Plants*, IAEA, 1969, p. 93.

EFFECT OF *IN VIVO* MUSCULAR STIMULATIONS
VI. Inhibition of Cardiac Proteolysis by the Electrical Stimulations of Leg Muscle

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ABSTRACT

The cardiac proteolysis was inhibited by the short term and prolonged *in vivo* muscular stimulations with an increase in the levels of soluble and structural protein fractions. The cardiac tissue appeared to have diverted the free amino acids towards the synthesis of both proteins and carbohydrates.

INTRODUCTION

PHYSICAL exercise is known to alter the metabolism of cardiac tissue[1–3]. Prolonged *in vivo* muscular stimulations[2–3] and conflict stress[4] increased the levels of oxidative enzymes of the tissues of the body and of myocardium respectively. Increased oxygen demand of myocardium[5], heart rate, arterial blood pressure and regional blood flow[6] are reported during physical exercise. Induced proteolysis is reported in various tissues of the body during heavy exercise[7] and repeated electrical stimulations[8]. Attempts to demonstrate changes in the protein levels of cardiac tissue during exercise led to contradictory results[4,9–10]. In the light of these conflicting statements an attempt is being made to understand the effect of short term and prolonged *in vivo* muscular electrical stimulations on the protein metabolism of cardiac tissue.

MATERIAL AND METHODS

Frogs, *Rana hexadactyla* (Lesson), were employed for the present investigation. Right gastrocnemius muscles of intact frogs were stimulated with electronic stimulator (INCO/CSIO Research Stimulator—Ambala) as described earlier[2], with a series of impulses (biphasic) of 5 V at a frequency of 2 pulses/sec for 30 minutes per day for one day in one batch of experimental animals and for ten successive days in another batch. The duration of each impulse was 100 ms, while the delay was 400 ms.

The cardiac tissue was isolated as usual for biochemical studies. Protein contents in the supernatant (water soluble) and the residue (water insoluble —structural) as obtained by the centrifugation of tissue homogenate at 3000 rpm for 30 min are estimated by the method of Lowry *et al.*[11]. Protease acti-