

## SUMMARY

The effect of ethyl methanesulfonate (EMS) - a mutagenic chemical on amoebae especially at defined phases of the cell cycle had been investigated.

1. Sensitivity of the cells to the action of EMS was found to be dose and cell cycle phase dependent where maximum sensitivity, in terms of cell lethality, was noted in mitotic and S phase cells.
2. Treatment of 0.5% EMS for 15 min was found to be the most suitable dose to carry out all the experiments.
3. Cell membrane was found to be greatly affected as was evident by the disorganisation of cell surface structure, revealed by SEM analysis. Cell detachment at early hours following EMS exposure and development of increased surface stickiness at later hours were also noted.
4. A change in the cell shape, cessation of cell motility, inhibition in the formation of pseudopodia along with the impairment in the process of phagocytosis were observed in the treated cells.
5. Delay in cell division was found to be a general effect after EMS treatment.

6. EMS induced cellular damage was noted as evident by cell cytolysis, cell fragmentation, formation of hyaline zone and appearance of hypervacuolization.
7. Three types of variant cells were produced after EMS treatment namely, mini cells; intermediate-sized-cells and giant cells among which only few mini cells were found to retain smaller size. The other cell types were either lethal or were regulated back to normal form. The giant cells were generally multinucleate.
8. Formation of altered form of cytoplasmic crystals was noted at later hours following treatment of the cells at MS phase.
9. Several cell organelles, like golgi bodies and mitochondria, were found to be disorganised after EMS exposure. Ribosomal particles were often observed as monomers through the cytoplasm.
10. The cell nucleus was found to be affected much after the treatment which showed nuclear swelling, fragmentation of the nucleus, formation of bizarre nucleus, shedding off the nuclear honeycomb layer, disorganisation of the nucleoli and chromatin materials, branching and fusion of the nucleoli. RNP helices were almost absent at early hours in the treated cell nucleus.

11. Cytochemical studies of the treated cells showed ununiform distribution of basophilia and distinctly less intensity of staining reaction of total protein moiety, bound lipids, PAS positive substances and alkaline and acid phosphatases.
12.  $^3\text{H}$ -thymidine incorporation study showed depression in DNA synthesis throughout the S period in ES phase treated cells. Proportionally more inhibition to DNA synthesis was noted in MS phase treated cells, compared <sup>to</sup> ES and LS phase treated cells.
13. Total cellular RNA synthesis, as assayed by  $^3\text{H}$ -uridine incorporation, was found to be inhibited to a great extent in MS phase treated cells and the depression in the synthetic activity/continued for quite long thereafter.
14. Protein synthesis as measured by  $^3\text{H}$ -leucine incorporation, was inhibited immediately following EMS treatment. Cell cycle experiments showed a continued inhibition of protein synthesis throughout the cell cycle in both ES and MS phase treated cells.
15. The most important finding of the present investigation had been the production of a mini amoeba mutant which appeared after treating the cells at ES phase. This mutant strain had been cultured in the laboratory

for more than two years and has undergone over 650 generations to date with unaltered mutant characteristics. The following features were encountered in mini mutants:

- (a) It has a cell cycle-phase-specific origin. The mini cells were emerged out from the parental cells by containing a comparatively smaller nucleus, which appeared after its fragmentation, along with some part of the cytoplasm.
- (b) The frequency of mini cell production was in the order of 9.4%.
- (c) These cells were considerably smaller in size and also contained a nucleus of smaller diameter, compared to parental cells.
- (d) Chilomonas were found to be the suitable food organism for mini cells as they could not survive after Tetrahymena feeding.
- (e) A great deal of alteration of the cell surface structure was evident from SEM analysis. These cells also developed a great deal of stickiness to the surface.
- (f) A change in the form of cytoplasmic crystals was evident.

- (g) The nucleus contained extensively dense nucleoli and had a highly dense and granulated nature of chromatin. Appearance of a layer of microfibrils around the nuclear boundary was observed.
- (h) The mitochondria had an anastomosing nature of cristae. The endoplasmic reticulum had vesiculated nature and were stubbed with ribosomes.
- (i) Duration of S phase and the pattern of DNA synthesis differed from the parental cells.
- (j) All the phases of the cell cycle were reduced and thus the entire cell cycle was shortened to a considerable extent as compared to control as well as the treated parental cells.