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MERCURY INDUCED STRUCTURAL ANOMALIES IN THE STOMATAL COMPLEXES OF ONION LEAVES

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Mercury induced anomalies in the organization of stomatal complex were analysed in *Allium cepa* v. Nasik N-53. Healthy bulbs were treated with seven concentrations (10^{-7} M-10^{-1} M) of mercuric chloride for 48, 96 and 144 hours and leaf samples were analysed for induced anomalies thrice, at a regular interval of one month each. The structural variations induced in the stomatal complexes were of two types: (1) those produced due to degeneration of guard cells, and (2) the others which were the result of developmental errors. The first kind of anomalies included stomata with one guard cell or without guard cells. Developmental errors gave rise to : a. one guard celled stomata with laterally placed stomatal pores, b. one guard celled stomata with centrally placed pores, c. stomata with unequal guard cells, d. stomata with guard cells separated by transverse wall, e. stomata with multinucleate guard cells, and f. stomata with three guard cells.

Key Words : Mercury; epidermal; structural anomaly; stomatal complex.

It is a well established fact that plants react in various ways to alterations or interference to different morphogenetic events. Some plant reactions can be visualized in the form of morphological and anatomical variations in tissues and organs. Foliar epidermis is one of such tissues which may respond rather quickly to different environmental pollutants and mutagens. While evaluating the genotoxicity of heavy metal mercury on certain crop plants, it's effect on the epidermal pattern was critically analysed. This communication deals mainly with the structural anomalies induced by mercury in the organization of stomatal complex of the leaves of *Allium cepa* cv. Nasik N-53.

MATERIALS AND METHODS

Bulbs of similar size were treated with seven molar concentrations (10^{-7} M - 10^{-1} M) of mercuric chloride for 48, 96 and 144 hours. For the treatments, the bulbs were put on small bottles filled with mercuric chloride solutions, so that only their lower most portion contacted the solutions. These bulbs, after thorough washing, were sown in polythene bags having a mixture of soil and manure. The control set was raised from untreated bulbs. Leaf samples, collected at regular interval of one month from each treatment set, were fixed in F.A.A. Due to conspicuous size difference between the leaves of different treatment sets, always the biggest leaf available was selected for the study, The epidermis was peeled out from the middle portion of the leaves, stained in Delafield's haematoxylin and mounted in glycerin. The main parameters analysed included, frequency distribution of stomata per mm², types and frequencies of stomatal anomalies, guard cell index (GCI) and area of stomatal pore. A parameter given the name response coefficient (RC) was calculated for inferring inhibitive and promoting effects of the treatments. GCI and RC were calculated using following formulae.

$$GCI = \frac{\text{no. of guard cells per mm}^2.}{\text{(no. of guard cells + no. of epidermal cells) per mm}^2.} \times 100$$

$$RC = \frac{\text{Value of treated set - value of control set}}{\text{value of control set}}$$

OBSERVATIONS

Bulbs treated with 10^{-2} M survived for a month only, hence only the first month leaf sample could be analysed in this treated set. Elliptical shaped normal stomata distributed between elongated epidermal cells were each comprised of two uninucleate guard cells surrounding the pore (Fig. 1). The frequency distribution of normal stomata and epidermal cells, GCI and area of stomatal pores in three consecutive monthly samples are given in table 1.

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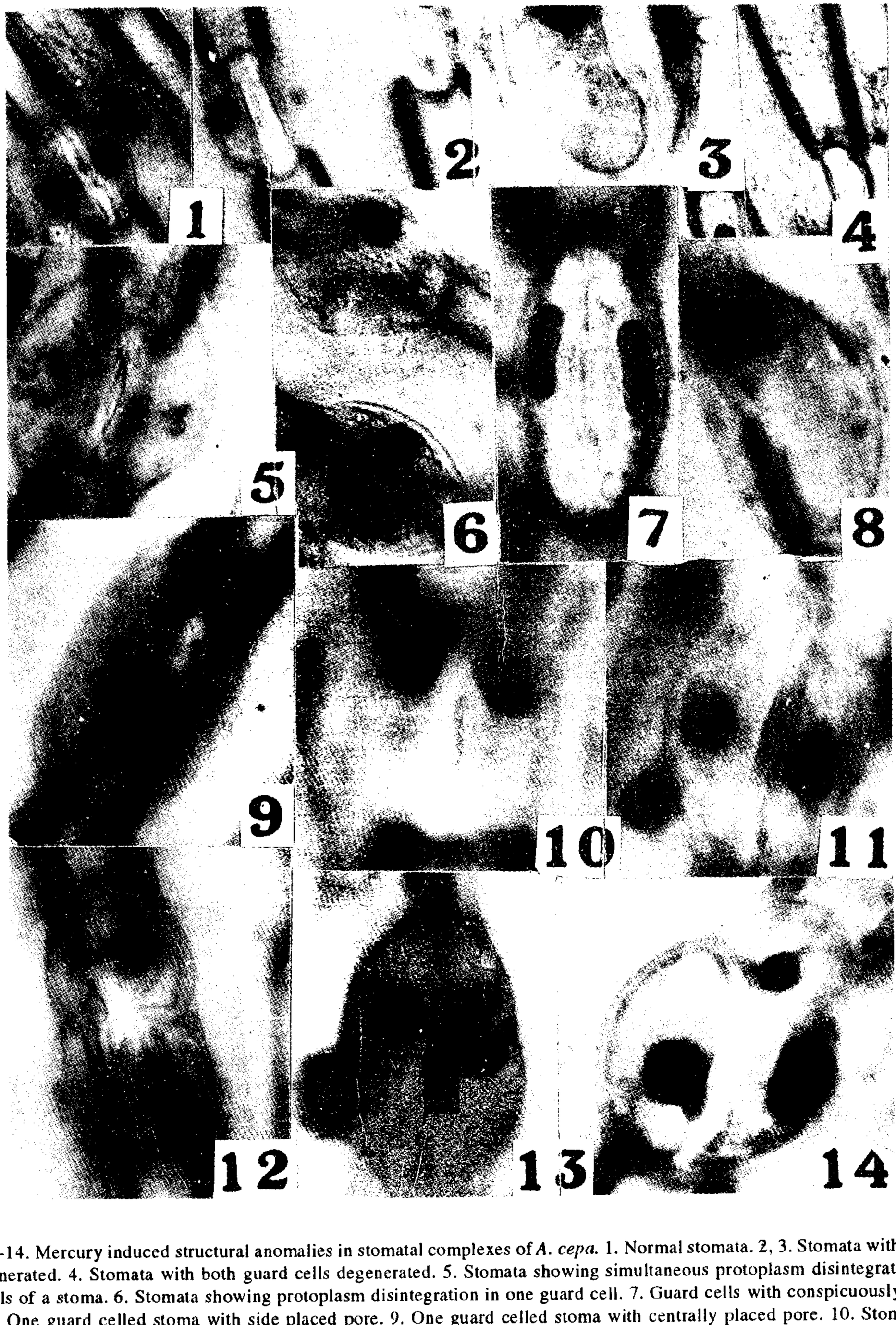


Figures 1-14. Mercury induced structural anomalies in stomatal complexes of *A. cepa*. 1. Normal stomata. 2, 3. Stomata with one guard cell degenerated. 4. Stomata with both guard cells degenerated. 5. Stomata showing simultaneous protoplasm disintegration in both guard cells of a stoma. 6. Stomata showing protoplasm disintegration in one guard cell. 7. Guard cells with conspicuously elongated nuclei. 8. One guard celled stoma with side placed pore. 9. One guard celled stoma with centrally placed pore. 10. Stoma showing dissolution of contact wall between guard cells. 11. Stoma with unequal guard cells. 12. Stoma with guard cells separated by transverse wall. 13. Stoma with binucleate guard. cell.

Table 1. Frequency distribution of stomata,epidermal cells, GCI and area of stomatal pores in control sets.

	Stomata per mm²	Epi. cells per mm²	GCI	Pore area µm²
Mon. I	128.31±6.10	253.52±3.29	50.30±0.56	74.80±0.19
	98.59-154.93	225.35-281.69	43.75-57.89	74.23-75.40
II	121.13±4.10	240.85±2.70	50.04±0.76	55.15±0.15
	96.59-140.85	225.35-253.52	45.16±54.05	54.73-55.64
III	140.85±7.71	280.28±3.38	50.13±0.74	54.81±0.11
	98.59-183.10	225.35-323.94	46.15-54.17	54.47-54.99

*Upper values refer to mean± S.E., lower values refer to range.

The values of RCs for frequency distributionof stomata and epidermal cells, GCI and pore area in treated set samples are graphically represented in figures 15-18. Although, the treatments with mercuric chloride stimulated the frequencies of stomata and epidermal cells in most of these sets, GCIs were decreased on the other hand. The stomatal pore area was significantly decreased in all the treated sets.

The control sets did not possess stomatal anomalies. Mercury induced structural abnormalities of the stomatal complexes were either due to degeneration of the guard cells(s) of mature stomata or were due to their anomalous development. The frequency of first type was much higher than that of other type (data not given here). Percent total stomatal anomaly in different treated sets, graphically presented in figure 19, clearly indicates the induction of significant amount of these in response to mercuric chloride treatment.

Guard cell(s) degeneration resulted into stomata with only one guard cell (Figs. 2,3) or without guard cells (Fig. 4). Since, the degeneration could only be recorded in the guard cells of mature stomata, treatments with higher concentrations like 10^{-1} M and 10^{-2} M for 96 and 144 hours, where stomatal maturation was inhibited, possessed either nil or highly reduced frequency of guard cell disintegration, as compared to other treatment sets. Disintegration of guard cells followed the expected pathway of cell degradation.The nucleus of the guard cell degenerated first followed by degradation of the cytoplasm and disintegration of wall except at the places where it contributed in pore formation. In many instances, the reminiscent of disintegrated wall could be seen around the stomatal pore. The protoplasm disintegration took place either simultaneously (Fig. 5) or one after the other (Figs. 2,3,6) in two guard cells. In many guard cells the nuclei became conspiciously elongated before degeneration (Fig. 7).

Anomalous development of stomatal complexes produced: a. one guard celled stomata with side placed pores (Fig. 8), b. one guard celled stomata with centrally placed pores (Fig. 9), this type of stomata, probably, also resulted through the fusion of the two nuclei of the two sister guard cells due to dissolution of contact wall portions not participating in pore formation (Fig. 10), c. stomata with unequal guard cells (Fig. 11), d. stomata with guard cells separated by transverse wall (Fig. 12), e. stomata with multinucleate guard cells (Fig. 13), and f. stomata with three guard cells (Fig. 14).

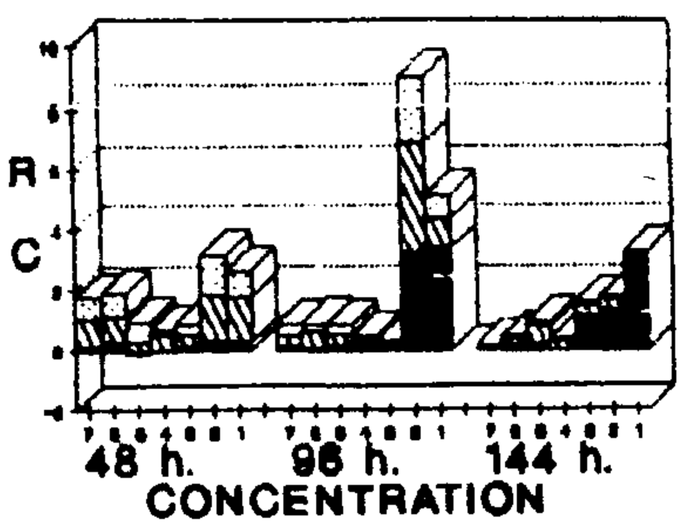
A. clear cut positive dose response correlation between the amount of effect induced and the concentration and duration of treatment was not available. However, concentrations higher than 10^{-4} M were producing more sever effects than the lower concentrations.

DISCUSSION

Increased frequencies of stomata and epidermal cells in many treated sets were because of the retardation of leaf elongation, expansion of epidermal cells and maturation of stomata.

Although, air pollution induced guard cells disintegration has been earlier reported by many workers including Bhiravmurthy and Kumar (1983), Bhiravmurthy *et al.* (1985), Black and Black (1979), Kulshrestha *et al.* (1980), Mishra (1982), Rajachidambaram and Krishnamurthy (1979), Rao and Ramayya (1967), Sen *et al.* (1972), Srivastava *et al.* (1980), Yunus and Ahamad (1979), Yunus *et al.* (1979) *etc.*, effect of water pollutants like mercury and other heavy metal compounds on the foliar epidermal traits have not been seriously worked out.

It was noticed during this investigation that the guard cells of only mature stomata could degenerate. The degeneration begins with nuclear disintegration followed by cytoplasm and wall disintegration. Breakdown of stomatal metabolism, either because of mercury accumulation in guard cells or because of mercury induced mutation(s) might be responsible for guard cell degeneration. The same explanation is also applicable to stomatal anoma-



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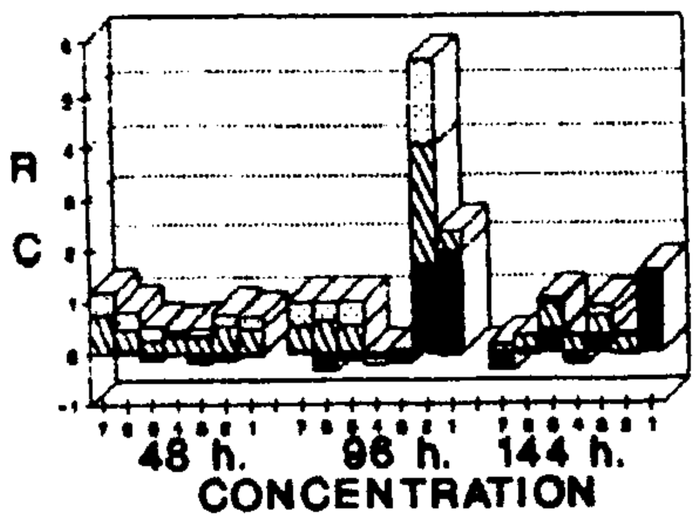

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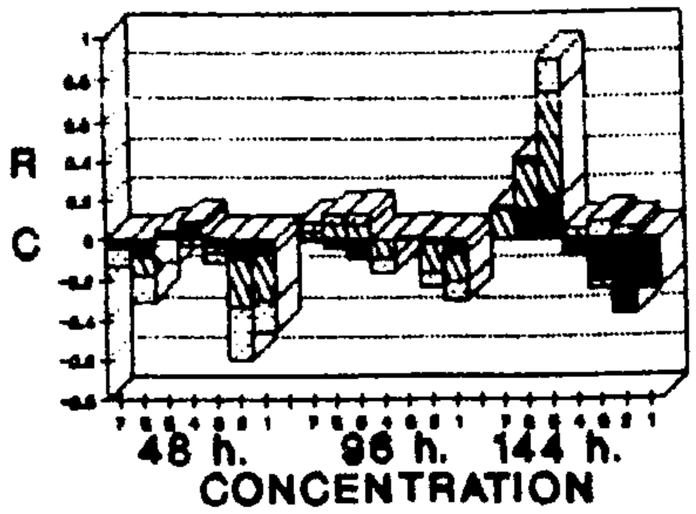

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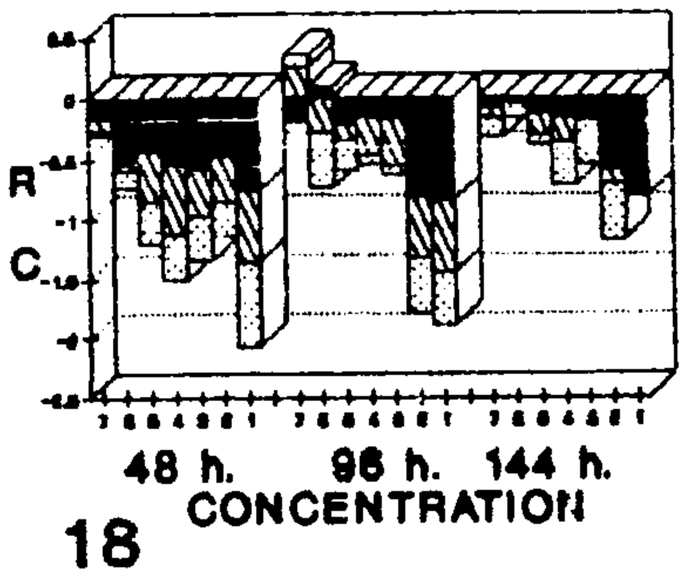

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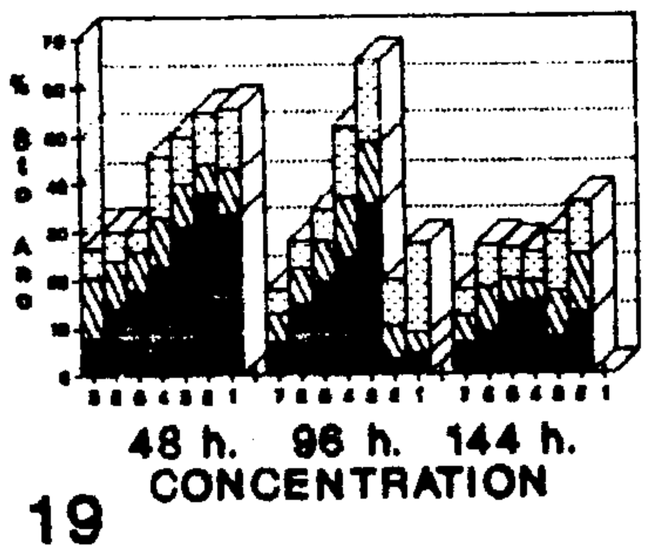

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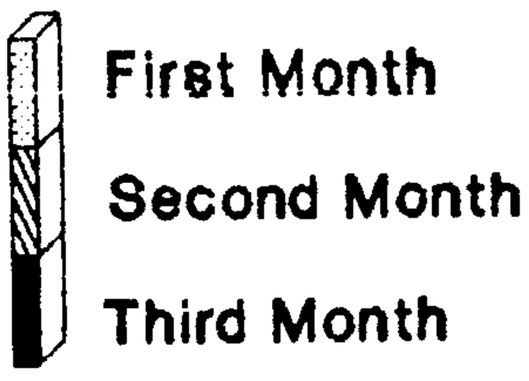

Figures 15-19. Graphical representations of the effect of mercury concentrations on the foliar epidermal traits of *A. cepa*. Figs. 15-18. RCs for frequency distribution of stomata and epidermal cells, for GCI and area of stomatal pores. Fig. 19. Total stomatal anomaly (7-1 = 10^{-7} M- 10^{-1} M concentrations).

lies resulting from induced developmental errors. Since, the mercury was given to ungerminated bulbs only for a limited period of time and the leaf samples were collected at least after one month of the treatment, mutation(s) appear more probable reason for induced stomatal anomalies.

The observed types of stomatal anomalies, produced because of developmental errors, are probably reported for the first time. Moreover, in all the stomata possessing these anomalies, stomatal pores were always present even when guard mother cell did not divide and differentiated into single guard cell. It appears more likely that meristimatic cells, destined to produce stomata are irreversibly programmed for producing pores and also possibly this programming is not coupled with programming responsible for dividing mother cell longitudinally. To the best of our knowledge, direct experimental evidences for the above conclusion are still awaited.

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