



Rats of all the groups were supplied with equal amount of food and water *ad libitum*. Upon gaining the body weight  $150 \pm 10$  g, rats from Gr. II and IV were exposed to repeated doses of MIC vapour (0.32 mg/L for 8 min for 5 consecutive days) in all glass whole body inhalation chamber under static condition. Control rats (Gr. I and III) were given fresh air under similar conditions. MIC was synthesized in our laboratory (5).

Six rats from each group were sacrificed by cervical dislocation at different post exposure days following the final exposure, i.e. immediately and on days 1, 2, 3, 7 and 14. The lung was excised immediately after sacrifice and 10% (w/v) homogenate was prepared in chilled KCl (0.15 M) using Potter Elvehjen homogeniser with ten up and down strokes. A portion of lung was fixed in 10% buffered formalin and processed for routine histopathological examinations.

The enzymatic activities of alkaline and acid phosphatases (ALP & ACP), reduced glutathione content (GSH) and total protein were assayed following the method of Wootton (6), Jollow et al (7) and Lowry

et al (8) respectively. The data were subjected to student's 't' test to measure the significant differences.

## RESULTS

Rats showed eye irritation, gasping and dyspnea following subsequent exposures of MIC vapour. No significant effect was observed upon food and water consumption and body weight.

Lung from control rats showed normal histoarchitecture (Fig. 1). Immediately following the final exposure, microscopical findings of biological significance were confined to the bronchioles in the form of inflammation, epithelial degeneration, desquamation, hyperactive secretory cells and regenerative hyperplastic and hypertrophic epithelial cells extending down to and including the terminal bronchioles (Fig. 2). The desquamated epithelial lining cells left basement membrane open at places showing development of submucosal fibrosis. Major areas of the alveolar septa were thickened with hypertrophied pneumocytes, edematous fluid with mononuclear cells and few polymorphs.

TABLE I : Effect of repeated exposure of MIC (0.32 mg/L) on the activities of alkaline and acid phosphatases (n moles phenol/min/mg protein) in lungs of rats of 20% and 8% dietary groups.

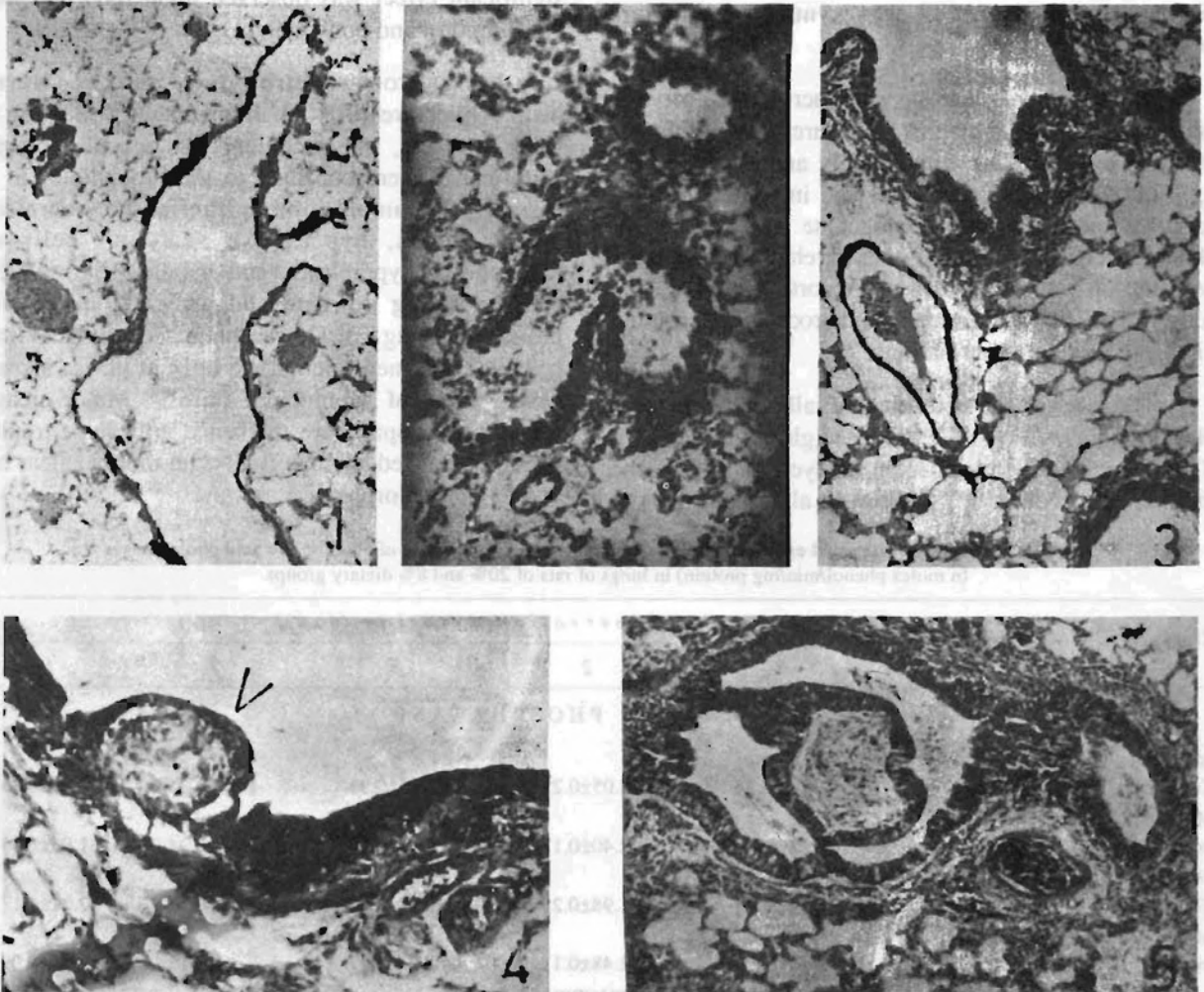
Groups	Immediately	Observation duration (days)				
		1	2	3	7	14
ALKALINE PHOSPHATASE						
20% Diet						
Control	1.98±0.21	2.07±0.25	2.05±0.21	2.13±0.33	1.87±0.25	1.79±0.23
MIC exposed	2.55±0.15*	2.77±0.11*	2.40±0.17	2.33±0.07	2.07±0.24	1.93±0.15
8% Diet						
Control	2.15±0.15	2.05±0.13	1.98±0.25	1.97±0.13	2.05±0.21	2.22±0.17
MIC exposed	2.87±0.23*	2.77±0.27*	2.48±0.11	2.24±0.27	2.20±0.15	2.33±0.09
ACID PHOSPHATASE						
20% Diet						
Control	1.18±0.22	1.19±0.15	1.27±0.25	1.35±0.25	1.33±0.40	1.39±0.27
MIC exposed	1.89±0.21*	1.79±0.17*	1.71±0.19	1.55±0.30	1.50±0.44	1.51±0.35
8% Diet						
Control	1.29±0.27	1.35±0.23	1.40±0.25	1.21±0.25	1.24±0.19	1.33±0.22
MIC exposed	2.14±0.22*	2.29±0.21**	2.18±0.22*	1.68±0.35	1.60±0.22	1.49±0.37

Values are mean±SE of six observations

\*P < 0.05; \*\*P < 0.02

On day 1, the frequency of the regenerating epithelial cells, hyperplasia and hypertrophic changes were enhanced which on day 2 was several layered thick protruding towards the lumen (Fig. 3). These lesions were more marked on day 3 and the intraluminal

fibroplastic projections became evident in some of the lumens (Fig. 4). On day 7 and 14, cellular changes aggravated further and the intraluminal fibroplastic tissue projections were very prominent and almost blocked the bronchiolar lumens (Fig. 5). Other cellular changes



- Fig. 1 : Section of lung from Gr. I, showing normal histoarchitecture of bronchioles and alveoli having normal epithelial lining cells. H and E  $\times$  96.
- Fig. 2 : Section of lung of Gr. IV showing hyperactive secretory cells, desquamated cells in the lumen, thickened alveolar septa, edema and emphysema immediately after the final exposure. H and E  $\times$  125.
- Fig. 3 : Section of lung from Gr. IV on day 2, showing aggravated hypertrophy and hyperplasia of bronchiolar epithelial lining, submucosal fibrosis with persisted edema and emphysema. H and E  $\times$  100.
- Fig. 4 : Section of lung from Gr. IV on day 3, showing hypertrophic and hyperplastic bronchiolar cellular changes, submucosal fibrosis (+) and intraluminal fibrous tissue projection (<). H and E  $\times$  125.
- Fig. 5 : Section of lung from Gr. IV showing hyperactive secretory cells, prominent intraluminal fibrous projection almost blocking the bronchiolar lumen, on day 12. H and E  $\times$  100.



TABLE II : Effect of repeated exposure of MIC (0.32 mg/L) on reduced glutathione (mg/g tissue) content in the lung of rats of 20% and 8% dietary groups.

Groups	Immediately	Observation duration (days)				
		1	2	3	7	14
20% Diet Control	3.83±0.22	3.97±0.30	3.75±0.29	3.79±0.25	3.83±0.27	3.96±0.23
MIC exposed	2.76±0.38*	2.78±0.37*	2.58±0.33**	2.78±0.33*	3.01±0.19*	3.20±0.25*
8% Diet Control	2.99±0.31	2.89±0.33	2.78±0.31	2.93±0.33	2.78±0.33	2.97±0.25
MIC exposed	1.90±0.30**	1.72±0.27**	1.45±0.19***	1.67±0.23***	1.71±0.21**	1.71±0.23*

Values are mean±SE of six observations

\*P < 0.05; \*\*P < 0.02; \*\*\*P < 0.01

still persisted, however, congestion of the parenchymal blood vessels and edematous changes were reduced. These lesions were prominent in protein deficient exposed rats. The activities of ALP and ACP elevated significantly ( $P < 0.05$ ) immediately following the last exposure and remained high till day 1 in normal dietary group while in protein deficient group, the level of ALP remained high upto day 1 and that of ACP upto day 2 (Table I). Table II reveals the effect of MIC on GSH content which reduced significantly in both the dietary groups immediately and remained low till day 14.

### DISCUSSION

The denudation of respiratory epithelium and subsequent regeneration of the tissue damage in the form of cellular proliferation and infiltration, mild edema, emphysema, peribronchial fibroplasia and intraluminal fibroplastic epithelial tissue projections observed in the present study reflect toxic effects of MIC and are in agreement with other reports (9, 10). The process of fibrogenesis as observed in the present study appears to be due to desquamation of bronchial epithelium followed by collection of fibrin and fibroblasts in the wound which on replication formed connective tissue mass and became covered by regenerating epithelium.

The initial elevation of ALP and ACP following MIC exposure may be associated with necrotic and

hyperactive effect on bronchial epithelial cells, macrophages and infiltrated monocytes. However, more so in protein deficient rats may be due to low ciliary activity of tracheo-bronchiolar region (11), which may have allowed more MIC to enter into the lung. The transudation of serum proteins in the edema fluid is another potential source of these enzymes.

The depleted level of GSH content following MIC exposure may be attributed to its high affinity for sulfhydryl group at physiological pH and its active involvement in the detoxification pathway of MIC. The MIC administered rats have been reported to form a chemically reactive glutathione conjugate [S-(N-methyl-carbamoyl) glutathione] (12). The formation of this metabolite may be a major factor behind the reduction of the glutathione content in the present study which also corroborate the reduction of blood glutathione levels in MIC exposed population of Bhopal.

MIC, thus, causes severe histometabolic dysfunction by affecting the respiratory surface area and has a positive correlation with malnourishment as far as its toxic potential is concerned.

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## DISCUSSION

The induction of respiratory epithelium and subsequent regeneration of the tissue damage in the form of cellular proliferation and inflammation and hyperplasia, as observed in bronchial epithelium, is a protective mechanism against the toxic effects of MIC and may be associated with other reports [9, 10]. The process of regeneration as observed in the present study appears to be due to regeneration of bronchial epithelium followed by collection of fibrous and fibrillar connective tissue which on regression formed connective tissue mass and become covered by regenerating epithelium.

The main objective of ALP and ACP following the exposure may be associated with necrosis and

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