Table 1 Effect of MIC on L-132 cell monolayers

<table>
<thead>
<tr>
<th>Concentrations of MIC (mg/ml)</th>
<th>CPE observed at different hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
</tr>
<tr>
<td>3.0</td>
<td>4+</td>
</tr>
<tr>
<td>0.3</td>
<td>3+</td>
</tr>
<tr>
<td>0.03</td>
<td>2+</td>
</tr>
<tr>
<td>0.003</td>
<td>—</td>
</tr>
<tr>
<td>0.0003</td>
<td>—</td>
</tr>
</tbody>
</table>

Degree of CPE: 4+ = 75-100%, 3+ = 50-75%, 2+ = 25-50%, 1+ = 10-25%, — = No CPE.

CPE consists of coagulation necrosis, shrinkage, rounding and detachment of cells.

A SIMPLE TECHNIQUE FOR ASSESSMENT OF IMMUNE-RESISTANCE OF HOSTS AGAINST GIARDIASIS

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GIARDIASIS, due to Giardia lamblia a common cause of malabsorption and diarrhoeal illness mostly in children has become a serious global problem, especially in view of the various immuno-deficiency symptoms. Giardiasis is a self-limiting infection in healthy humans and animals. Both humoral and cellular immune processes are reported to play an active role during giardial infection. Peritoneal macrophages and polymorphonuclear leukocytes (PMNL) are closely associated with amoebic infection. Phagocytic competence of these cell-types is adversely affected by virulence of the pathogen thus determining the fate of the pathogen in the host. Reduced, cellular PMNL-cytotoxicity was noticed in giardiasis. Based on this principle, an in vitro test method has been developed to determine the immunocompetence of human-PMNL in tackling G. lamblia cells during giardiasis.

A drop of blood from the suspect patient was dispensed directly over a microscope slide and incubated at 37°C for 1 hr under humidity. Blood clot was flushed-out under a jet of phosphate buffer saline (PBS) at pH 7.2. Twenty-four hour old axenically grown trophozoites of G. lamblia (5 x 10⁶ cells/ml) were centrifuged at 900 x g for 15 min. washed and suspended in modified complete Diamond’s axenic medium. Suspension was dispensed over the PMNL-monolayer and incubated at 37°C under humidity for 10-15 min. Following interaction, the monolayer was dried, fixed with methanol and stained with giemsa for 2 hr. Excess stain was removed by normal saline, dried and examined under oil immersion microscope.

The leukocyte population on the monolayer (figure 1) constituted exclusively of PMNL without any contamination with other blood cell-types. Compared to the conventional methods of preparation of peripheral PMNL, employing Ficoll Hypaque centrifugation, the present method appeared simpler. Similarly the population of G. lamblia-trophozoites used for interaction appeared healthy and motile (figure 2).

26 May 1986; Revised 25 July 1986

On interaction, normal PMNL, derived from healthy individual, lyzed the giardial cells (figure 3); however, the situation got reversed on interaction with PMNL of chronic giardiasis patient, where the leukocytes were lyzed by *G. lamblia* (figure 4).

These observations are corroborated from the findings of Gitter *et al* for *E. histolytica* and to Smith *et al* for *G. lamblia*. Bos and Van de Griemd demonstrated the virulent *E. histolytica* killed 50% peritoneal PMNL (10 cells/amoeba) within 15 min of interaction. Similarly Guerrant *et al* found that less virulent amoebae were rapidly surrounded and damaged by the healthy human-PMNL.

Apparently virulence is a valuable parameter that must be accounted for predicting the mode and the fate of the interacting pathogen with the host. Thus the chronicity of giardiasis may be interpreted in terms of the reduced PMNL-cytotoxicity of the host.

The present technique, which involves 6–8 hr operation, may offer information regarding the immune status of the host by *in vitro* interaction of *G. lamblia* and peripheral PMNL of the giardiasis patient. It may be postulated that the reported method, which requires minimum equipment, cost and operational skill, may be adopted as a preferable tool for the routine field or clinical trials to ascertain the immuno-competence of the host against giardiasis.

The authors thank Dr M. M. Dhar, Director for his keen interest. Financial assistance from ICMR to RKS is thankfully acknowledged.

7 June 1986; Revised 17 July 1986

EFFECT OF DIMECRON ON SOMATIC CELLS OF ALLIUM SATIVUM L

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Environmental chemicals, biocides, are known to induce cytological abnormalities in plant and animal cells. In many cases the cytological abnormalities caused by biocides are very similar to those induced by mutagenic agents \(^1\)–\(^8\), and hence screening of biocides for their mutagenic potential is gaining in importance. Dimecron is mostly used as a systemic insecticide on various crop plants. Therefore, it was considered desirable to test the mutagenic potential of dimecron on plants, as this information will be helpful in understanding the mechanism of cytological damage as well as its implication on environmental pollution. In the present investigation an attempt is made to study the effect of dimecron on the course of mitosis and on the mitotic chromosomes of Allium sativum L.

Healthy roots of A. sativum were treated at room temperature with 0.05, 0.1, 0.15 and 0.2% of dimecron in distilled water for 2, 4 and 16 hr. Simultaneously, adequate controls were maintained under identical conditions in distilled water. The root tips of both treated and controls were excised and fixed in 1:3 acetic alcohol. Cytological preparations were made using aceto-orcein. The cytological observations were recorded on 500 cells selected from ten different root tips of each treatment.

The data presented in Table 1 show that the mitotic indices at various concentrations were consistently low in all treatments and the decline is greater at higher concentrations (0.15 and 0.2%). After treatment with 0.05% for 16 hr the fall in the mitotic index was 39.2% but it was 71.2% after 2.0% concentration for 16 hr. A similar decline was observed both in short and long durations (Table 1). The abrupt fall in the mitotic indices soon after dimecron treatment indicates that the preceding G2 stage affected many cells entering the mitosis and suggests the chronic effect on all or some of the preceding stages \(^3\).

The spectrum of cellular responses included scattering of chromosomes (C-mitosis, Figure 2) and the daughter chromatids held together only at the centromeric regions referred to as diplochromosomes. This is possibly due to the inactivation of the spindle apparatus and consequent delay in the division of centromere \(^9\). The spectrum of anomalies also includes sticky metaphase followed by disturbed anaphase giving rise to tripolar and tetrapolar cells. Nuclear pycnosis and chromosome clumping were observed in considerable number. Cytogenetic effects such as fragmentation (Figure 1), breaks (Figure 3) and bridges were observed at anaphase and telophases. The chemical also induced chromosomal contraction at all concentrations and at longer durations (16 hr) of study. The insecticide treatment also resulted in bi-, tri- and tetraneicate cells indicating the inhibition of cytokinesis.

Differences in nuclear pattern, size and shapes were observed in the cells treated for longer duration. In many cells the nuclei were seen located eccentrically. Non-synchronization of chromosomes