water and even viable seeds lack germination[9]. Such seeds are called hard-seeds. The hardness of seed coat is to some extent attributed to the presence of intact macrosclereid layer[10] and usually requires a pre-germination treatment such as heat or scarification. Chemical treatment of seeds with acetone or sulphuric acid breaks the barrier to water movement into the macrosclereid layer[11] resulting in seed germination. It is now documented that seed dormancy in *Sesbania* is caused by the macrosclereid layer which is impermeable to water. Both *Sesbania bispinosa* and *S. punicea* have seed coat structure typical of legumes but the former shows tightly packed macrosclereid layer and this compactness makes the seed coat less permeable to water[11]. Interestingly many seeds of both the species showed 'rust' coloured testa where the macrosclereid layer was interspersed with triangular cavities[11]. Such seeds were always permeable to water. The integrity of macrosclereid layer appears vital to seed coat impermeability. In *Cyamopsis tetragonoloba* the macrosclereid layer reveals triangular cavities resulting in speedy germination of seeds that were soaked in water. The seeds of *Crotolaria retusa* and *C. spectabilis*[3] have compact macrosclereid layer that acts as a barrier to water movement. In these two taxa a cut with a razor blade at the chalazal end without damaging the embryo and the endosperm before soaking in water for a few hours was necessary for seed germination. Treatment which cause the seed coat permeable to water apparently do so by causing a change in the structure of the testa[12]. Thus the changes that bring about permeability of water in the legume seeds appear to be due to the change in structuralism of the macrosclereid layer.

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ACTION OF ANTIHISTAMINE ON SOMATIC CELLS OF *ALLIUM SATIVUM* L.

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CHLORPHENIRAMINE maleate is successfully used as an antihistamine. Information on its effects on somatic and meiotic cells of plants and other systems is meagre. Hence, an attempt is made in the present investigation to study the effects on the course of mitosis and on the mitotic chromosomes of *Allium sativum*.

Healthy roots of *A. sativum* were treated at room temperature with 0.5, 1.0, 1.5 and 2.0% concentrations of chlorpheniramine maleate in warm distilled water for 1, 2 and 4 hr. Adequate controls were simultaneously maintained under identical conditions in distilled water. Roots were harvested from treated and control plants, immediately after treatment and after 24 hr recovery period, and fixed in 1:3 acetic-ethanol. Cytological preparations were obtained by using aceto-orcein stain[1]. The frequency of mitotic figures and aberrations, induced by the drug was determined by scoring about 2000 cells from each treatment.

Mitotic indices at various concentrations were consistently low in all treatments, the decline getting sharper with higher concentrations. After treatment at 0.5%, the fall in the mitotic index was 22% but 46% after 2.0% treatment. A similar decline was observed both in short and long durations *i.e.* 30% in 1 hr, 35% in 2 hr and 38% in 4 hr treatment (table 1).

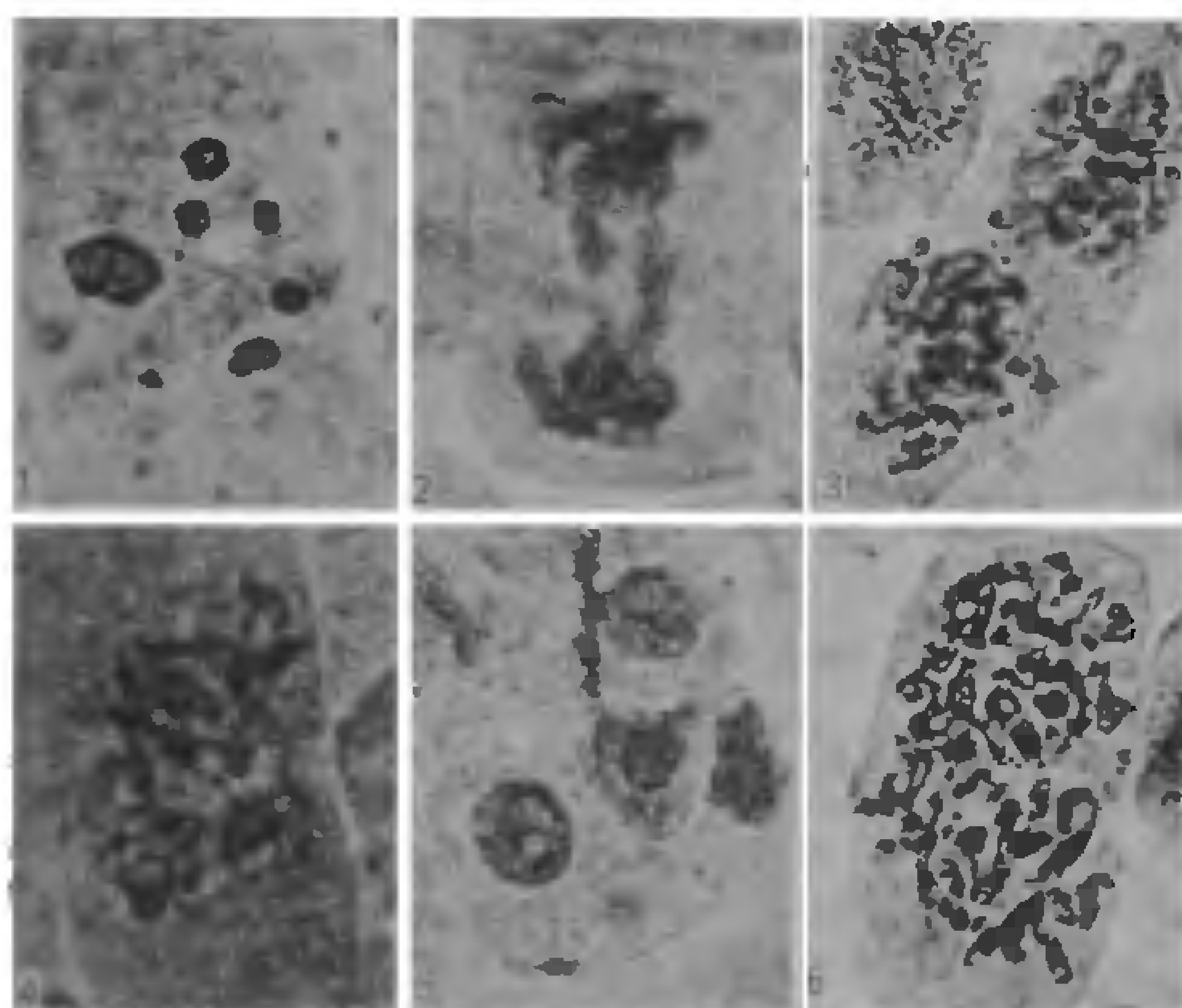
The steep fall in the mitotic indices soon after 0.5% treatment, indicates that the preceeding G_2 stage affected many cells from entering mitosis and is suggestive of the chronic effect on all or some of the preceeding stages[2]. The low mitotic indices and the linear relation of mitotic decline with higher concentrations, also occurring with colchicine and vinblastine[3], seem to be due to a slow rate of cell cycle progression through mitosis rather than inhibition of any particular stage[4].

Table 1 *Mitotic indices following treatment with chlorpheniramine maleate*

Concentration in Percentage	Duration of treatment in hours			Mean
	1	2	4	
Control	12.5	7.9	4.2	8.2
0.5	10.0 (20)	6.1 (23)	3.2 (24)	6.4 (22)
1.0	9.4 (25)	5.4 (32)	2.7 (36)	5.8 (31)
1.5	8.3 (34)	5.0 (37)	2.4 (43)	5.2 (38)
2.0	7.5 (40)	4.1 (48)	2.1 (50)	4.6 (46)
Mean	8.8 (30)	5.2 (35)	2.6 (38)	

Values in parentheses are % of decline in the mitotic indices

The spectrum of chromosomal aberrations observed was scattering of chromosomes, formation of bridges (figure 2), chromocentric nuclei *i.e.*, intranuclear clumping of chromatin, resulting in differential staining (figure 1), binucleate cell formation and micronuclei. Disruption in the orientation of anaphase groups (figure 4) and precocious movement of chromosomes were also prominently observed. Tetranucleate cells (figure 5) and bimitosis *i.e.*, biprophase (figure 3) bimetaphase and polyploid cells (figure 6) were observed after recovery.



Figures 1–6. Showing various mitotic abnormalities induced by chlorpheniramine maleate on *Allium sativum*. **1.** Cell showing extreme condensation of chromatin material at interphase × 4200, **2.** Sticky double bridge at anaphase × 4200, **3.** Cell showing late biprophase × 3000, **4.** Multipolar grouping of chromosomes at anaphase × 4200, **5.** Cell showing tetra-nucleate condition × 3000, **6.** Polyploid cell × 3000.

The 0.5% concentration seemed to be minimal in effect, resulting in the scattering of chromosomes. Other concentrations caused an array of other responses. Spindle inhibition was more from 1.0% concentration onwards. There was a tendency towards excessive chromosomal condensation with increasing durations and inhibition of cytokinesis with increased concentrations. Micronuclei and chromocentric nuclei were induced at higher concentrations only. Aqueous recovery for 24 hr yielded a mixture of normal and double prophase and metaphase nuclei. Polyploid cells were also observed.

The scattering and binucleation may be due to the inhibition of cytokinesis and is so far known to be caused by aminopyrin[5], caffeine[6], pyramidon[7] and thuringiensin[2]. On recovery, these binucleate cells also showed prophases and metaphases, exactly as after caffeine treatment[6].

These observations indicate that the microtubular system of the spindle phragmoplast and cell plate is impaired and that the impact could be so enormous as to affect the inhibition of the spindle microtubules even of the succeeding cell cycle[8]. Nevertheless, it may be presumed that the microtubule disfunction could be due to the inhibition of DNA dependent RNA polymerase[9] or to the direct action on the tubular proteins, as reported for vinblastine[10,11].

Differential staining of the nuclei observed in the present study, and also caused by vinblastine[3] suggests the intra-nuclear clumping of chromation making the nuclei look like chromocentric nuclei of animal cells. The micronuclei that occurred, appeared to be chromosome residues of spindle disfunction as evidenced by some of the abnormalities and by the absence of any clastogenic effect[12,13] in spite of the occasional observations of bridges.

During the present investigation, the drug mainly simulates the mitotic effects of colchicine, vinblastine, caffeine, chloralhydrate and thuringiensin besides its C-mitotic effect. As these chemicals are known to be mutagenic or antineoplastic, it seems highly probable that chlorpheniramine maleate also has similar mutagenic or antineoplastic potentiality.

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HAEMAGGLUTINATION BY CERTAIN BACULOVIRUSES

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AMONG the various entomopathogens, baculoviruses, comprising of nuclear polyhedrosis virus (NPV) and granulosis virus (GV) seems to have a great potential for the control of insect pests. Nearly 1271 host-virus records have been reported[1]. However, before considering the widespread field application of such viruses, a detailed knowledge of their characteristics appears essential. Haemagglutination is a property of a number of animal viruses[2]. Since the discovery by Hirst[2], that influenza virus could cause haemagglutination, this reaction has provided an effective tool for the study of certain basic properties like invasive mechanism of animal viruses. But in the case of insect viruses, haemagglutination was generally unsuccessful[3], until Miyajima and Kawase[4] showed that cytoplasmic polyhedrosis virus (CPV) and NPV of silk worm, *Bombyx mori* agglutinated chicken, sheep and mouse erythrocytes and by Shapiro and Ignoffo[5] with NPV of *Heliothis zea* on chick red blood cells (CRBC). In several cases, the adsorption of the viruses to cells has been mediated through the same receptors that are involved with the agglutination of vertebrate erythrocytes[6]. Hence, an attempt has been made to find out the haemagglutinating property of certain baculoviruses and the results of the study have been presented here.

The NPVs infected larvae of *Spodoptera litura*, *Heliothis armigera* and *Corcyra cephalonica* and GV of *Pericallia ricini*, were triturated in a solution containing 0.14 M sodium chloride and 0.115 M sodium citrate and 0.001 M 1-phenyl-2-thiourea. The triturate was spinned through several layers of muslin cloth. Both polyhedral inclusion bodies (PIB) of NPVs and capsules of GV obtained from those larvae were purified by repeated differential centrifugation and saline water washes and stored at 5°C and used as and when needed. For haemagglutination test, pooled fresh blood from several chicks were drawn into Alsever's solution, centrifuged, and the pellets of RBCs were washed thrice with normal saline. Washed cells were suspended in saline to obtain a 0.5% suspension. Two-fold serial dilutions of virus suspensions were made from a stock containing 1.1×10^6 PIB/ml in saline in perspex plate and to each well equal quantity of erythrocytes suspension was added and incubated at room temperature. The highest dilution of inclusion body suspensions producing 100% haemagglutination was considered as one HA unit. In the case of doubtful results, the perspex plates were incubated at 5°C overnight and observed for HA on the next day. Both normal saline solution and erythrocyte suspension mixture and extract of healthy insect tissue were kept as controls.

It is evident from the result (table 1) that the NPVs of *S. litura* and *H. armigera* and GV of *P. ricini* had the property of agglutinating chick erythrocytes whereas NPV of *C. cephalonica* was negative. The above results with NPV of *S. litura* confirmed the earlier report of Wani *et al*[7]. But the titre obtained in this case is of 1 : 80 which is comparatively low. This may be due to

Table 1 *Haemagglutination response of chick erythrocytes by certain baculoviruses*

Sample	Response	Titre
NPV of *S. litura*	+ ve	1:80
NPV of *H. armigera*	+ ve	1:160
NPV of *C. cephalonica*	− ve	—
GV of *P. ricini*	+ ve	1:80