ture. They were hydrolyzed 24 h after fixation, with 1N HCl at 60 °C for 2 min. This was followed by several washings with distilled water. They were then stored in 45% acetic acid, until squashes were made in acetocarmine from the apical 2 mm. The percentage of cells in mitosis in each experiment were determined on the basis of a minimum of 1000 cells. Observations on the percentage of mitotic abnormalities were recorded.

*Effect of tobacco smoke on general morphology.* In untreated seedlings the cells had well-defined nuclei and the mitotic division was normal in 95% cells. Cells exposed to the tobacco smoke showed little evidence of injury. Only a few cells treated with tobacco smoke were damaged, as evidenced by blebbing of the cytoplasm and slightly pycnotic nuclei in the stained preparations. Treated cells, in general, were highly vacuolated.

*Effect of tobacco smoke on mitotic index.* In controls, about 9% of the cells were in mitosis, there being a variation from 7–10% in various samples. Treatment with tobacco smoke led to a decrease in mitotic index; magnitude of the effect varied with the number of puffs to which seedlings were exposed. Control cells showed a mitotic index of 0.09 while roots treated with 25 puffs showed an index of 0.05. Table I summarizes mitotic indices of onion root-tips exposed to different numbers of tobacco smoke puffs.

*Effect of tobacco smoke on chromosomal abnormalities.* Tobacco smoke also induced mitotic abnormalities. The percentage of irregularities increased with increasing number of puffs. Five 35-ml puffs induced abnormalities in only 10% of dividing cells while 10, 15, 20 and 25 puffs caused 38%, 47%, 58% and 60% irregularities, respectively, in the dividing cells. In general, 'stickiness' of chromosomes (Figure 1), and lagging chromosomes (Figure 2) in the equatorial plate were observed.

NAKANISHI et al.[3] while working with human epithelial lung cells treated with cigarette smoke condensate observed a decrease in average chromosome number to 76, from a modal value of 77 for the chromosomes in untreated cells. This finding was attributed to the appearance of dicentric chromosomes in some of the passages. BOUCHARD and MAY[13] observed an increased number of mitotic abnormalities in mouse lung fragments that had been bathed for 24 h in a smoke condensate solution. Later investigations[14–16] also found that cigarette smoke condensate has profound influence upon mammalian cells. Yet with a very few exceptions[17–20] which are perhaps best exemplified by chronic granulocytic leukemia in humans, the observed chromosomal anomalies commonly found in cancer cells show no consistent pattern. The present investigation is another approach used to ascertain the feasibility of utilizing plant tissues as a quick and sensitive bioassay for tobacco smoke. Vacuolization of the cytoplasm, pycnotic nuclei and other mitotic abnormalities were seen 24 h after exposure to cigarette smoke. Other mitotic irregularities include occasional 'stickiness' of chromosomes at metaphase (Figure 1) and bridging at anaphase and telophase (Figure 2). Thus, the present investigation clearly demonstrates that one can detect similar cytological responses in plants and animals exposed to tobacco smoke. A study of tobacco smoke and its constituents on plant tissues might lead to a better understanding of their mechanism of action. We are currently investigating the effect of different subfractions of tobacco smoke and results will be reported subsequently.

*Zusammenfassung.* Es wurden Untersuchungen der Wirkung des Tabakrauches auf mitotische Fehler bei *Allium-cepa*-L.-Sämlingen gemacht und dabei eine Abnahme des mitotischen Indexes und induzierte mitotische Missbildungen gefunden.

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## Influence of Prior Exposure of Male Khapra Beetle (*Trogoderma granarium*) to Female Sex Pheromone on Their Mating Ability

It has been suggested that by saturating the field atmosphere with female sex pheromone or its synthetic analogues, male orientation to female could be effectively disrupted[1–3]. Experimental proof in support of this has been provided by GASTON et al[4]. They maintained in the field a high concentration of *Trichoplusia ni* pheromone and showed that under these conditions males were unable to orient towards virgin females held in traps. Sensory adaptation and/or absence of definite odour trail have been suggested as possible reasons for this phenomenon. It is however, also possible that the males were exhausted as a result of high excitation due to stimulation by the pheromone, and were unable to fly towards the trapped females. It is then likely that such exhaustion would impair their mating ability. We therefore investigated the effect of possible pheromone-induced fatigue on the ability of males to mate, using the khapra beetle, *Trogoderma granarium* Everts as test insect.

Five-day-old virgin males were confined in air-tight glass specimen vials (8 × 2.3 cm ⌀) each containing a filter paper disc impregnated with 0.2 ml of pheromone extract[5] (equivalent of 50 females' secretion over a 2-day-period). After 48 h these males were removed from the vials and paired in batches of 10 with an equal number of 1-day-old virgin females and were held in plastic containers (6 × 6 cm ⌀) with broken wheat as oviposition medium. Untreated males similarly paired served

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as controls. A week after the death of all females, the larvae present in the medium were scored. In 3 of the 5 replicates in treatment, there were no larvae and in the remaining ones their number was much less compared to controls (Table).

Since an unmated female is always infertile, the absence of larvae in 3 replicates suggests that the males had failed to mate even though the females were in the vicinity. It is therefore reasonable to infer that failure to mate was probably due to exhaustion resulting from excitation during previous exposure to the pheromone. A few matings discernible in the other 2 replicates suggest that either some of the males were not exhausted, or they recovered in due course.

Mating ability of *T. granarium* males previously exposed to female sex pheromone

| Replicate | No. Larvae | |
|---|---|---|
| | Treatment | Control |
| 1 | 0 | 280 |
| 2 | 135 | 308 |
| 3 | 0 | 207 |
| 4 | 39 | 234 |
| 5 | 0 | 208 |

*Résumé.* L'exposition prolongée des *Trogoderma* mâles vierges au phéromone sexuel femelle a réduit considérablement leur capacité d'accouplement, quoiqu'il y eût des femelles à proximité. Ce fait résulte probablement de l'épuisement des organes excités pendant l'exposition au phéromone sexuel.

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## PRO EXPERIMENTIS

## Autoradiography of Whole-Mounts of Isolated Ectoderm of the Body Column of *Hydra*. A New Method[1]

So far studies concerned with cell migration, cellular turn-over, and structural aspects of axial gradients in *Hydra* have been performed according to the classical histological techniques of using serial sections. Besides being time consuming, this procedure does not exclude erroneous quantitative estimations, since the possibility of counting the same cell twice on neighbouring sections cannot be avoided, especially in the case of radioactive labelling.

The method proposed serves the same purposes, but since it works with whole-mounts of the entire ectoderm of the body column, the cellular situation does not need to be reconstructed. Cells and groups of cells appear in their natural and undisturbed two-dimensional configuration. In isolating the ectoderm from the endoderm, we have improved and refined the technique described earlier[2–4] and have made it available for autoradiographic studies.

The experimental animals, *Hydra attenuata* Pall.[5], were grown in artificial culture medium[6]. The hypostome and the tentacles of the polyps were amputated just below the tentacle crown. The remaining body was transferred to a slide previously coated with a thin film of egg albumin. In order to split open the tubular body of the *Hydra*, the tip of a watch maker's forceps was pushed longitudinally through the gastral cavity. The body wall was then lengthwise sliced open by rubbing the loop-like tip of a wolfram needle against the edge of the tip of the forceps. The opened body wall was then laid out on the surface of the slide, so that the ectodermal layer was in direct contact with the slide surface, while the endoderm faced upward.

The split open body was anaesthetized by adding a drop of MS 222 (0.02 g/10 cm³) and Novocain (0.5 g/10 cm³) previously mixed in a proportion of 5:1. 10 minutes afterwards the body wall was completely anaesthetized and had reached its maximal degree of two-dimensional extension (various other drugs tested failed to give satisfactory results, because the body wall, instead of expanding, remained contracted or shrank when alcohol and acetic acid were later added to separate the endoderm from the ectoderm).

The detachment of the endoderm from the ectoderm occurred after the addition of a few drops of a mixture of alcohol and acetic acid (2 cm³ 70% alcohol + 2 cm³ 5% acetic acid + 15 cm³ distilled water). This mixture gently dissolves the mesogloea so that the endoderm could be carefully removed with the help of a wolfram needle, portion by portion, without harming the ectoderm. Afterwards

Distances (μm) of the nuclei of different ectodermal cell-types from the mesogloea measured on histological cross-sections (7 μm) of the body column of *Hydra attenuata*

| Nuclei of: | *n* | Average distance (μm) from the mesogloea (standard deviation) |
|---|---|---|
| Epithelio-muscular cells | 70 | 14 ± 7 |
| Interstitial-cells | 209 | 16 ± 9 |
| Stenoteles: | | |
| Nematoblasts | 76 | 14 ± 8 |
| Nematocytes | 30 | 15 ± 10 |
| Desmonemes: | | |
| Nematoblasts | 60 | 11 ± 7 |
| Nematocytes | 45 | 5 ± 4 |
| Isorhizas: | | |
| Nematoblasts | 10 | 23 ± 12 |
| Nematocytes | 30 | 28 ± 18 |

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