Arrest of somatic cells at G_2 by Sevin (pesticide)

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Summary. The treatment of onion root meristems with the pesticide Sevin [carbaryl (1-naphthyl n-methyl carbamate)] induces an accumulation of interphase nuclei with a larger diameter, and a subsequent decrease in the index of smaller nuclei. It is concluded that Sevin depresses mitosis by arresting cells at G_2 without affecting DNA synthesis.

Sevin [carbaryl (1-naphthyl n-methyl carbamate)] is a commonly-used pesticide for the control of bugs, thrips, beetles and grasshoppers common on fruit and crop plants. Pesticides are known to give rise to cytogenetic hazards[2], both for target and nontarget organisms[3]. Sevin, a carbamate pesticide, has been found to be cytotoxic and to produce various chromosomal and nuclear abnormalities like spindle disturbances, lagging chromosomes, micronuclei formation, chromosome and chromatid bridges, large cell/nuclei formation, polyploidy, etc. in plant cells[4–11]. Besides, Sevin has been shown to be radiomimetic for barley[10–12]. As a pre-requisite for a proper knowledge on the mode of action of this pesticide at cellular and sub-cellular levels, the stage of the cell-cycle at which it is effective must be determined. A survey of the literature reveals that such an approach has so far been largely neglected. Amer[4] concluded that Sevin arrests cells at prophase and metaphase stages, and does not affect the rate of mitosis. Carbaryl is known to break down into hydroxycarbamates and other intermediates and affect DNA synthesis, and it thereby produces chromosomal abnormalities[2]. The results of the present study disagree with the above mentioned contentions[2,4] and suggest that cells are arrested by Sevin at G_2, and that DNA synthesis is not affected.

Materials and methods. Vigorously-developing 2- or 3-day-old healthy root tips of locally purchased onion (*Allium cepa*[L.]) bulbs grown in distilled water were used. The root tip cells were treated continuously for up to 12 h with 0.25% and 0.50% aqueous solutions of Sevin (formulated with 50% active ingredient; Union Carbide, India), prepared at room temperature. The controls were maintained in distilled water. Following the desired treatment, the root tips were fixed in acetic-ethanol (1:3), and later stained with aceto-orcein-(N) HCl mixture (9:1). They were squashed in 1% aceto-orcein solution. The diameters of interphase nuclei were measured at about × 450 magnification using a calibrated occular micrometer. Each nucleus was measured twice and the average measurement calculated. Data from measurements of more than 200 nuclei were pooled for each set of experiments.

Results and discussion. In squash preparations, the nuclei of the interphase cells of the meristematic zone have a circular outline. For the present purpose, therefore, the diameter of nuclei has been taken as reflecting their volumes. The diameter of interphase nuclei ranged between 12 and 30 µm. In the present investigation, as can be seen in the table, the interphase nuclei have been grouped into 5 categories on the basis of their diameter. The table shows the percentage of interphase nuclei with varying diameters in control and treated preparations. In control preparations, 12–18 µm nuclei account for 59%, 20–22 µm nuclei for 36%, and 24–30 µm nuclei for only 5% of the population of the interphase nuclei. The populations of the treated nuclei, on the other hand, show a different pattern. Following all the treatments, there is a remarkable increase in the percentages of the larger (24–30 µm) nuclei with a subsequent decrease in the index of the smaller (12–18 µm) nuclei. For example (see table), the population of interphase nuclei, after 12 h of continuous treatment with 0.25% Sevin, have 14% nuclei with 12–18 µm diameter, 50% with 20–22 µm diameter and 36% with 24–30 µm diameter compared with the control values of 59%, 36% and 5% respectively. Thus, the treatments induce an accumulation of bigger interphase nuclei; the longer the treatment, the greater the accumulation.

In our earlier study[8], Sevin was found to be mitodepressant. Continuous treatments with Sevin solutions up to 27 h revealed that depression in the mitotic index continues with a simultaneous accumulation of cells at interphase.

It is well established that the relative DNA values are positively correlated with nuclear size, as has been shown in the interphase nuclei of shoot[13] and root[14] meristems of different plant species[3], including *Allium cepa* L.[3], the present test material. Hence, in the present study, the gradual increase in the percentage of larger nuclei represents a continued accumulation of G_2 cells (4C DNA value), rather than G_1 cells (2C DNA value). On the other hand, a gradual slump in the index of smaller nuclei suggests that cells pass through G_1 and S without any hindrance before getting arrested in G_2. It may, however, be mentioned here that the G_2 arrest following 12 h of continuous treatment is partial. Further experiments (unpublished) have suggested that with the blockage of cell division following 27 h of continuous treatment, more than

Treatment	Interphase nuclei (%) Nuclear diameter 12–14 µm	16–18 µm	20–22 µm	24–26 µm	28–30 µm
Control	11	48	36	4	1
3 hours					
0.25%	4	32	49	13	2
0.50%	11	35	39	11	4
6 hours					
0.25%	5	23	44	24	4
0.50%	5	37	35	20	3
9 hours					
0.25%	2	15	53	25	5
0.50%	9	23	48	12	8
12 hours					
0.25%	1	13	50	30	6
0.50%	9	25	47	12	7

81.5% of arrested interphase cells are in G^2. The conclusion that Sevin induces mitotic depression by arresting cells in G^2 without affecting DNA synthesis is, therefore, inescapable. The arrest of cells in G^2 further suggests that this pesticide interferes with the cell cycle at that stage.

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Muscle crossbridge action in excitation and relaxation

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Summary. Pulse-propagation measurements on a muscle stimulated into tetanus show that the stiffness develops earlier and starts to relax later than the tension. It is concluded that the myosin heads which move towards the actin filaments during excitation become mechanically attached to them.

X-ray diffraction experiments have shown that when a muscle is stimulated the crossbridges swing out from the myosin filaments towards the actin filaments, returning to their original state when stimulation ceases[2]. However, an unexpected feature was revealed by more recent experiments[3,4] with time-resolution of the order of 10 msec, namely that the crossbridges move out more rapidly than the rate at which tension is developed and reach their final distribution well before the tension reaches its maximum value. Yagi et al.[5] have also shown that at the end of a tetanic stimulation there is a marked discrepancy between the fall of tension and the return of the myosin heads. Such discrepancies cannot be explored on the basis of the X-ray data alone as these measure only the proportion of crossbridges which are near to the actin filaments without necessarily being attached to them[6]. Yagi et al. therefore proposed that mechanical experiments such as stiffness measurements might resolve the issue. We now report pulse-propagation measurements which show that the stiffness of a stimulated muscle also reaches a maximum well before the tension is fully developed, and thus support the view that the crossbridges which move transversely outwards actually become attached to the actin filaments.

Methods. We used a pulse-propagation technique to obtain a direct measurement of the propagation velocity of a mechanical pulse over the central 30 mm (approximately), of a muscle specimen. Experimental details of this technique have been given elsewhere[7]. The stiffness of the specimen is calculated from the measurements as the product of the density and the square of the propagation velocity. There is considerable dispersion of the square-wave input pulses as they travel along the muscle but the measurements are made solely on the leading edge of the pulse, which propagates with a velocity of the order of 100 msec. The uncertainty in the arrival time of the transmitted pulse is of the order of 10 μsec. This is much smaller than measured relaxation times (reciprocal rate constants) for the initial recovery of stretched muscle fibers[8] so that the stiffness measured is a purely elastic or 'instantaneous' stiffness.

In the present work fiber bundles approximately 40 mm long from the sartorius muscle of the toad, *Bufo marinus*, were pinned horizontally between a tendon and the pelvic bone, in Ringer's solution maintained at 5 ± 1 °C. The pulse transmitting and receiving crystals were brought into contact with the specimen over the central 30 mm of its length, so that the compliance of the tendon and mountings plays no direct part in the result. However, these 'external' compliances do permit the muscle to contract by several percent during stimulation, an important difference from the truly isometric experiments carried out with the spot-follower of Huxley and his co-workers[9].

Results. In the figure, a, the solid-line curve shows the rise in tension as a specimen is stimulated into tetanus; the dashed curve shows the corresponding increase in stiffness above its level in the resting state. The curves are normalized to their values in the steady state of tetanus. When the muscle is soft the accuracy of the velocity measurements is too low to decide whether or not the stiffness and tension start to rise simultaneously. However, it is clear that the stiffness starts to increase proportionately more rapidly than the tension.

Furthermore (in contrast to an earlier, less accurate experimental result[7]) it reaches its final tetanic value well ahead of the development of maximum tension. This result parallels the X-ray diffraction observations and raises the problem of why the tension is still incompletely developed and takes approximately a further 200 ms to reach its tetanic level.

In relaxation, as the figure, b, shows, the stiffness decline is close to a mirror image of its development. The stiffness remains approximately constant for 150 ms until the tension has fallen by 10%. Optical measurements indicate considerable heterogeneity inside the muscle at this time, some sarcomeres expanding while others are contracting. At about 150 msec the stiffness starts to decrease rapidly indicating that a net detachment of crossbridges has begun. After about 250 msec, corresponding to the inflexion in the tension curve, the situation is dominated by the simultaneous expansion and contraction in different regions of