

Maximum reduction in the number of fertile eggs was observed when the flies were treated on the 7th day after eclosion (figure 1).

The identity of the stages at which mortality occurred during the peak period of sensitivity (6th and 7th days after eclosion) is shown in figure 2. While considerable numbers of embryos failed to complete embryogenesis in 6-day-old flies, this effect was not noticed when the flies were treated on the 7th day after eclosion. At this stage a large proportion of the mortality was due to inhibition of hatching of fully developed embryos. On both days of treatment a significant number of 1st instar larvae failed to molt to 3rd instar. There was no further mortality associated with larval-pupal and pupal-adult molts.

The peak sensitivity period of flies reported in this study coincides with the time of ovulation. In this respect these results parallel those reported for *Pyrrhocoris apterus*³, *Hayalophora cecropia*⁴, and *Drosophila melanogaster*¹⁰. Arrest of embryogenesis was significant only if the eggs were exposed to IGR just before ovulation; if the eggs were already ovulated at the time of IGR application (7-day-old flies) embryonic development was not affected, but hatching of fully developed embryos was blocked. Many of these embryos that hatched as 1st instar larvae also died before molting to 3rd instar (figure 2). Similar results of differential sensitivity of different stages of embryos was also observed in *Drosophila melanogaster*¹⁰.

The insensitivity of the flies to IGR during the period of oocyte growth explains the absence of any effect on fecundity. Reduction in the total number of eggs produced per female was reported only for those insects which were sensitive to IGR during the period of oogenesis^{5,11}. In *Sarcophaga bullata* maximal sensitivity was observed only during the period of ovulation, that is after the completion of egg maturation and therefore there was no reduction in the fecundity of the flies.

It is clear from these results that ZR-2646 acts as a juvenile hormone mimic for *Sarcophaga bullata* with 20 µg per fly causing significant ovicidal effects.

Total number of mature eggs produced per female after topical application of ZR-2646*

Age of flies at treatment (days after eclosion)	Acetone 5 µl	ZR-2646 5 µg	ZR-2646 20 µg
1	102	100	98
2	110	92	95
3	98	96	105
4	108	101	112
5	100	104	96
6	96	94	104
7	98	108	106

* Average from 50 flies for each treatment.

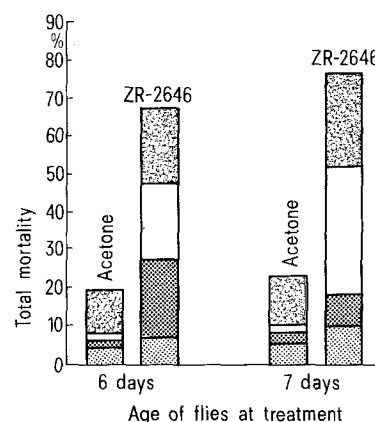


Fig. 2. Mortality of embryonic and postembryonic stages from treatment of female flies with 20 µg of ZR-2646. The unhatched fully developed embryos and hatched 1st instar larvae were cultured on beef liver. The undeveloped and poorly developed eggs were dechorionated and examined under phase contrast microscope. 1st and 2nd instar larvae; fully developed embryos; poorly developed embryos; undeveloped eggs.

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Satellite cells in denervated muscles

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Summary. It is known that, in a denervated striated muscle, the satellite cells multiply by mitotic division. A liaison between these satellite cells and the Schwann cell in front of the post-synaptic membrane in denervated frog muscle has been observed. It is probable that such cell connections help in the subsistence of the Schwann cell in a denervated muscle.

During the course of experiments on denervated muscles (rectus internus major and sartorius) of *Rana esculenta*, we observed by electron microscopy the multiplication of mononucleated cells designated as 'satellite cells' by Katz² and Mauro³. It is known that these satellite cells are capable of mitotic division, and that multiplication results from denervation⁴⁻⁶. During the 2nd month after denervation, the activation of satellite cells is manifested by an

increase in their size and organelles. There is an expansion of endoplasmic reticulum and Golgi complex; ribosomes and pinocytotic vesicles are in abundance. At this stage of denervation, these cells are seen multiplying, and often 2-3 of them are aligned one after the other between the basal lamina and sarcoplasmic membrane (figure 1). It seems that, after proliferation, these satellite cells are capable of protracting themselves from the original site of multipli-

tion, thus separating from the parent muscle. They can separate from the muscle fibre without detaching from each other completely and are observed in the intercellular spaces as a continuous line.
The Schwann cell, which has engulfed the axon terminal within 1 week after denervation, is present in front of the post-synaptic membrane at this stage of denervation^{7,8}. Its

connection with the cells described above are of particular interest. In figure 2, one after the other, 2 cells are connected to the Schwann cell by thin cytoplasmic bridges. It is difficult to get sections showing these cell connections, due to the fact that they are thin and wavy. Therefore, it seems probable that the satellite cells which are often seen overlying the sites of neuromuscular junctions, are usually con-

Fig. 1. 38 days after denervation. 3 satellite cells (asterisks) are lying between the sarcoplasmic membrane and the basal lamina. Mu, muscle fibre. M. rectus internus major. $\times 16,000$.

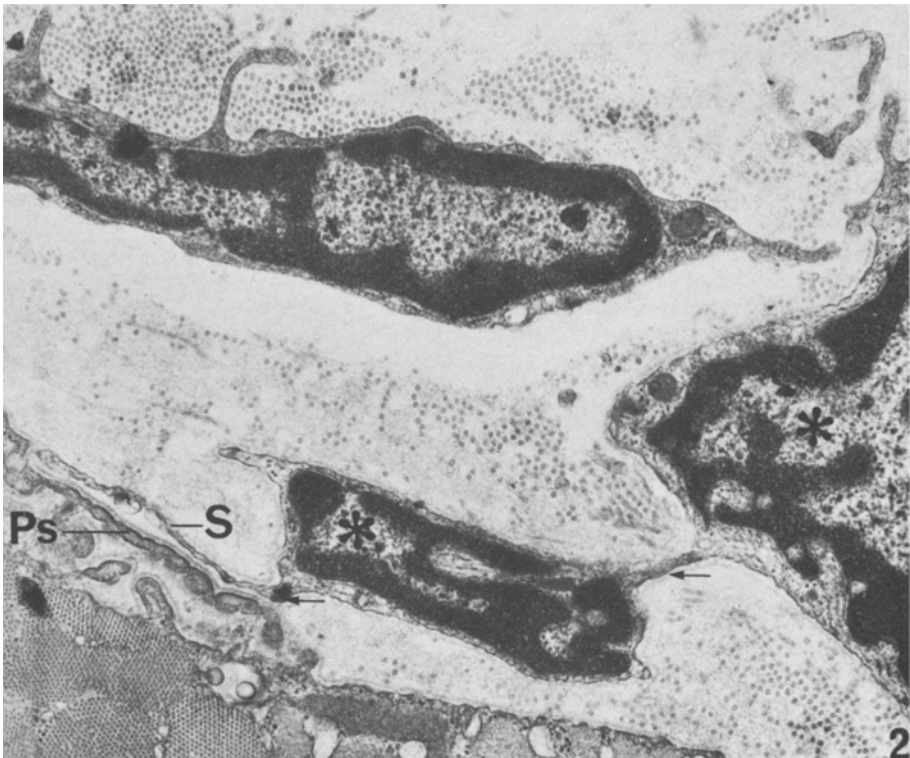
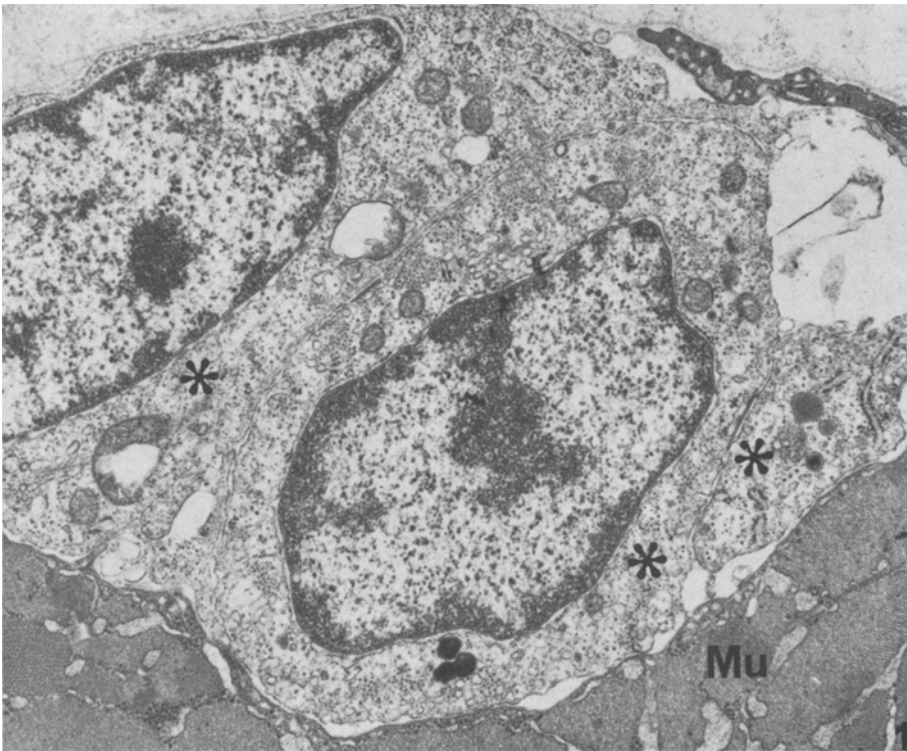


Fig. 2. 68 days after denervation. A Schwann cell (S) and the 2 satellite cells (asterisks) are connected by thin cytoplasmic bridges (arrows). Ps, post-synaptic membrane. M. Sartorius. $\times 20,000$.

nected with the Schwann cell by such thin cytoplasmic passages. What may be the functional significance of these cellular liaisons, is difficult to discern. A simple hypothesis is that satellite cells supply the Schwann cell with products by which the latter can subsist for longer periods, in front of the post-synaptic membrane, in a denervated muscle.

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Variations in DNA content

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Summary. DNA content in the active nuclei of various plant organs varies from organ to organ. The amount of DNA in the shoot tip is the least, as compared to that of other organs. The amplification of DNA in the differentiation of organs from the shoot meristem has been suggested.

The importance of DNA amplification in differentiated tissue has been claimed by various authors¹⁻³. It has been suggested that in plants there is a mechanism of phylogenetic increase of nuclear DNA through duplication, repeats and generative polyploidy. In absence of such a mechanism, an ontogenetic increase of nuclear DNA, as a prerequisite for differentiation, is adopted by the plants through endomitotic division³. The chromosomal behaviour in differentiated tissue has been cited as an example of dynamic behaviour of chromosomes^{4,5}.

In course of quantitative studies by us on in situ nuclear DNA, even in meristematic cells of different organs, variation in content has been observed⁶. As the cytological analysis shows their clear diploid chromosome content, these variations could not be attributed to difference in chromosome number.

There are, however, records of differences in amount of DNA in different organs, but such differences have mostly been reported in vitro involving polyploidy and aneuploidy^{7,8}. No systematic attempt towards quantitation of the amount of DNA from the actively dividing meristematic regions of the organs has been given. In view of the fact that variation in DNA content has been observed through in situ analysis, the present investigation was planned to make a systematic study of quantity of DNA of nuclei from different organs from various plant tissues. For an accurate analysis, quantitation through cytophotometry at in situ level from cytological smears, as well as from nuclei after extraction from tissue, has been preferred.

The work was confined to the following species of plants – *Pisum sativum*, *Pisum sativum* var. B-22, *Lens esculenta*, *Trigonella foenum-graecum*, *Nigella sativa*, *Hordeum vulgare* and *Aloe vera*.

Seeds were germinated in potted soil. When the roots were about 2 cm long, meristematic and differentiated zones (1 mm and 2–3 mm from the tips respectively) were cut out separately. For the isolation of nuclei, the material was kept in 5% citric acid for 1 h to soften the tissue. Acid was decanted and the material was washed with buffer solution (10 mM Tris-HCl, 5 mM CaCl₂, 5 mM MgCl₂, 0.25 M sucrose). Then 1 drop of mercaptoethanol was added to the material and it was homogenized with mortar and pestle and then with glass homogenizer with the buffer solution. The homogenate was then run through 2 layers of nylon cloth to remove the debris and then layered over 40% glycerol and centrifuged for 1 h at 10,000 rpm. The supernatant was discarded, the residue was suspended in 1.5% citric acid + 0.25 M sucrose, layered over 1.4 M sucrose, and centrifuged at 5000 rpm for 25 min. The nuclear pellet thus obtained was suspended in 0.25 M sucrose.

For cytophotometry (isolated nuclei), materials were fixed overnight in glacial acetic acid-ethanol mixture (1:2), treated with 5 N hydrochloric acid at 12–14 °C for 50 min–2 h, rinsed in distilled water and treated with Schiff's reagent for 2 h. Then the nuclei were isolated following the method already mentioned. Slides were prepared from the supernatant and mounted with 0.1% glycerol and sealed.

Table 1. Amount of DNA/nucleus (in situ) ($\times 10^{-12}$ g \pm SE)

Species	Root meristem in metaphase	Differentiated zone	Shoot meristem in metaphase
<i>Pisum sativum</i>	6.9 \pm 1.12	9.1 \pm 0.82	5.5 \pm 1.37
<i>Pisum sativum</i> var. B-22	5.2 \pm 0.91	8.0 \pm 1.41	3.7 \pm 1.16
<i>Lens esculenta</i>	4.4 \pm 0.76	6.1 \pm 1.0	3.3 \pm 0.91
<i>Trigonella foenum-graecum</i>	4.7 \pm 0.06	6.8 \pm 0.06	4.1 \pm 0.09
<i>Nigella sativa</i>	6.5 \pm 0.96	8.6 \pm 1.16	5.7 \pm 1.34
<i>Hordeum vulgare</i>	5.7 \pm 1.65	8.0 \pm 2.11	3.9 \pm 1.38
<i>Aloe vera</i>	4.2 \pm 0.93	8.5 \pm 1.39	4.0 \pm 0.98

Table 2. Amount of DNA/nucleus (extracted) ($\times 10^{-12}$ g \pm SE)

Species	Root meristem	Differentiated zone	Shoot meristem
<i>Pisum sativum</i>	6.1 \pm 1.39	7.0 \pm 0.98	4.2 \pm 0.55
<i>Pisum sativum</i> var. B-22	4.4 \pm 1.13	6.0 \pm 0.92	3.7 \pm 0.65
<i>Lens esculenta</i>	4.3 \pm 0.79	4.5 \pm 0.88	3.0 \pm 0.52
<i>Trigonella foenum-graecum</i>	3.9 \pm 0.12	6.5 \pm 0.09	2.1 \pm 0.06
<i>Nigella sativa</i>	6.0 \pm 0.92	7.8 \pm 1.89	3.6 \pm 1.23
<i>Hordeum vulgare</i>	5.4 \pm 1.11	6.8 \pm 1.06	–
<i>Aloe vera</i>	3.7 \pm 1.67	6.5 \pm 1.74	–