

raised with the help of a bent glass seeker and its branches were cut off.

A circular and longitudinal incision were made on the skin over the tendo-achillis (T-A) and GM respectively in order to peel off the skin over this muscle. The T-A below the ankle joint was cut off and the tendon along with the freed GM was lifted upto the knee joint. The head of the tibia and the lower end of femur were cut off. The freed SN with spinal attachment in one side and the muscle close to the knee joint on the other side were lifted from the body and placed in normal amphibian saline and used for in vitro study.

For study in vivo, the pithed toad was placed on myographic board with dorsal surface up and the muscles and nerves were exposed as previously. Freed tendon of the muscle was hooked by the pins attached to the short arm of the lever and the knee joint was fixed to the myographic board by the pin. The head of the tibia and lower end of femur were not cut off as done in isolated NMP.

**Results and discussion.** A simple-routine method developed for the NMP was experimented in our laboratory during the last 10 years. This preparation was found to be more advantageous than other existing methods<sup>1-3</sup> in certain respects. Since the approach was made on the dorsal surface of the animal, there was minimal chance of blood loss. In the previous method VA was made. As a result, the abdominal viscera came out with risk of damage. The contents of the rectum and urinary bladder might come out while bisecting the pelvic girdle. These waste products may disturb the sensitivity of the SN. This risk is overcome in the present method, and the time required for complete preparation is not more than 5 min. Study of NMP can be made in vitro and in vivo with and without circulation. Moreover, the same animal can be used for other purpose also (dissection of digestive system, urinogenital system etc.) as the abdominal viscera was not disturbed. These advantages are not feasible in the previous methods<sup>1-3</sup>.

## Gibberellin and Nucleic Acid Metabolism during *Zea mays* Fertilization

Y. LESHEM, HADASSAH SCHLESINGER and RINA PEELY<sup>1</sup>

Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel), 1 December 1975.

**Summary.** Either by direct GA supply or by release of glycosidic bound GA, pollination causes partial opening of double-stranded DNA in somatic maize kernel tissues, as evidenced by increased Tm profiles. This phenomenon is associated with enhanced RNA and protein production.

In lower organisms the act of fertilization triggers RNA activity and enhances amino acid incorporation, while in higher mammalian eukaryotes these promotive effects are experienced somewhat earlier in the sexual cycle, i.e. upon ovulation<sup>2</sup>. The effect reported here indicates that in higher plants the act of fertilization and/or pollination induces similar changes and that the process may be triggered by the phytohormone gibberellic acid (GA).

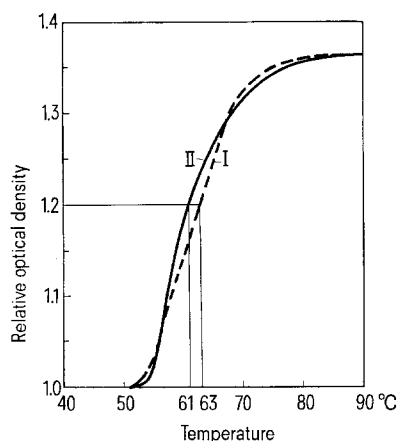
**Methods.** Cell experiments were carried out upon *Zea mays* cv Jubilee kernels from plants growing in sandy loam soil at the Beit Dagan Agricultural Experimental Station in central Israel. The two developmental stages compared (referred to henceforth as stage I and stage II) were just prior to pollination when the silk stigmas were about to reach the receptive stage to pollen, and subsequently

36-40 h later. This period is the maximal time reported<sup>3</sup> for the pollen tube to extend and fertilize the ovule.

Biochemical parameters measured were thermal denaturation (Tm) profiles<sup>4</sup> of extracted DNA<sup>5</sup> (indicating extent of single stranded regions); extraction<sup>6</sup> and bioassay<sup>7</sup> of endogenous GA-like activity, and determination of RNA<sup>8</sup> and protein<sup>9</sup> content. All extractions were performed on 200 g (fresh weight) samples of whole kernels. Each extraction was performed in 4-6 replicates and results statistically assessed by analysis of variance: least significant differences (LSD) are expressed at the  $p < 0.05$  level.

**Results.** The Figure compares Tm profiles of the 2 developmental stages. From this figure it is apparent that in stage II following ovule fertilization the Tm profile is 2°C lower than in stage I. This indicates increment of single stranded DNA regions.

The Table provides a comparison of other measured parameters. As seen from this table, in stage II there is a significant rise in free-GA as well as considerable increase in RNA and protein.



Comparison of Tm profiles of DNA extracted from *Zea mays* cv Jubilee kernels at 2 successive stages. I, just prior to pollination; II, upon fertilization.

<sup>1</sup> Acknowledgments: The authors wish to thank Dr. Y. WURZBURGER for her aid and advice in extraction and bioassay procedure. This research was financed by a grant from the Bar-Ilan Research Council.

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**Discussion.** As evidenced by Tm profiles shown in the Figure, fertilization results in a greater proportion of single stranded DNA. This phenomenon is accompanied by significant increase in free GA-like activity. In the light of the reports of FELLEBERG<sup>10</sup>, BAMBERGER<sup>11</sup> and KESSLER and SNIR<sup>12</sup> that in vitro GA causes DNA 'opening', it is postulated that in the maize kernel the DNA Tm profile changes following fertilization are GA triggered. The observed effect may be the result either of direct binding of GA to DNA as reported above or, alternatively, by GA-induced enhanced membrane permeability which possibly provides 'unwinding' enzymatic agents greater accessibility to the DNA.

Comparison of endogenous GA-like activity, RNA and protein levels in *Zea mays cv* Jubilee kernels at successive developmental stages

Developmental stage	Free GA <sup>a</sup>	Glycosidic bound GA <sup>a</sup>	RNA content <sup>b</sup>	Protein content <sup>c</sup>
I	0.260	3.860	0.255	1.550
II	0.430	3.260	0.322	2.060
LSD <i>p</i> < 0.05	0.110	0.140	0.059	0.380

I and II – as in the Figure. Figures presented are 4 replicate means expressed as O.D. units at <sup>a</sup>560 nm, <sup>b</sup>259 nm and <sup>c</sup>660 nm as determined on a recording Unicam UV-spectronic photometer Model SP 800 B.

The GA performing this function may stem from conversion of bound to free GA. The Table indicates that, with a decrease of the former, an increase of the latter is manifested. A further GA source, not necessarily excluding the above, may be the pollen, since BARENDSE et al.<sup>13</sup> have reported that pollen grains contain considerable amounts of plant growth hormones including GA. It thus seems that in the maize kernel the partial 'unwinding' of DNA may result in greater template activity and this may account for the observed increase in RNA and protein levels (Table).

These effects are primarily in the kernel tissue excluding the egg cell(s) and the ovule sac which are the site of fertilization, since preliminary determinations indicated that DNA content of the embryo (at either stage) was negligible as compared to that of whole kernels. Furthermore it has been shown<sup>14</sup> that during the initial stages following fertilization the developing ovule contains only a small number of nuclei. This implies a general stimulatory effect of fertilization taking place in a comparatively minute locus of generative tissue upon metabolism of somatic tissue.

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Effects of Denervation and Decentralization upon Taste Buds

J. A. DONOSO<sup>1</sup> and P. ZAPATA<sup>2</sup>

Departamento de Neurobiología, Universidad Católica de Chile, Casilla 114-D, Santiago (Chile), 25 August 1975.

**Summary.** Denervation of vallate papillae results in failure of tactile and gustatory reception at a time when impulse conduction in the distal stump of the glossopharyngeal nerve is still unimpaired; delay of receptor deficit depends on axon length between receptor and axotomy sites; taste buds disappear by 10 days. Decentralization, through intracranial rhizotomy, does not modify lingual receptors structure or function.

Taste buds differentiation and maintenance depend on their connection with gustatory nerves. Those buds of the vallate papillae are innervated by the glossopharyngeal nerves, and they degenerate shortly after nerve severance<sup>3,4</sup>. The present work was a study of some structural and electrophysiological changes in taste receptors after neural damage at different levels.

Experiments were performed on adult cats under pentobarbital anaesthesia. One or both glossopharyngeal nerves were transected either: a) at its immergence into the pharyngeal wall, b) at its exit from the jugular foramen, c) at the emergence of its roots from the brain stem, approached through a craniotomy of posterior fossa (Figure 1). Levels *a* and *b*, separated by ca. 20 mm, are peripheral to the petrossal and superior ganglia, and thus severance at these sites produce 'denervation' of taste buds, while damage at level *c*, central to sensory ganglia, produces 'decentralization' of receptor organs. Electrophysiological recordings of peripheral stumps of both glossopharyngeal nerves (severed at different levels or one intact as control) were performed between 15 h and 84 days after the initial operation, and followed by fixation and staining of the vallate papillae for histological studies.

Glossopharyngeal nerve activity was elicited by mechanical (rubbing the posterior third of the tongue

with a plastic probe) and chemical (jets of citric acid, quinine or NaCl solutions directed towards the vallate papillae) stimuli. While impulses in mechanosensory fibres are generated directly by deformation of nerve terminals, sapid substances act on epithelioid receptor cells which in turn excite synaptically chemosensory fibres.

Tactile and gustatory responses disappeared between 22 and 30 h after denervation (transection at levels *a* or *b*), tactile responses usually persisting for 1 or 2 h after chemical stimuli were no longer effective. This confirms an earlier report<sup>5</sup> that generator activity in nerve terminals fails at a time when conduction of nerve impulses electrically-elicited is still preserved. Indeed, we found that the compound potential and conduction velocity in the peripheral stump of transected glossopharyngeal nerves were normal at least up to 36 h after operation. The early failure of receptor function may be correlated

<sup>1</sup> Fellow of the Chilean Commission for Scientific and Technological Research (CONICYT).  
<sup>2</sup> Work supported by grant 1/71 of the Catholic University Research Fund. Thanks due to Dr. F. E. SAMSON JR. for reviewing the manuscript and to Ms. LUZ-GLORIA OPORTUS for technical assistance.  
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