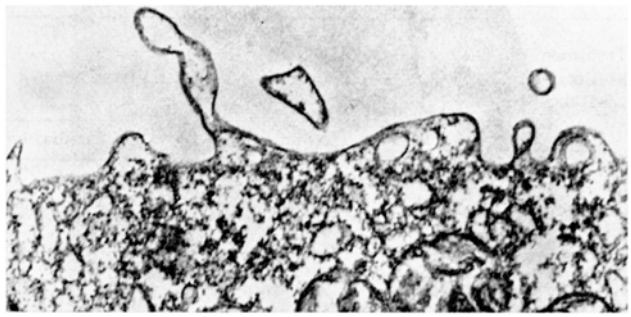
A Fertilization Reaction in *Ascidia nigra*: Formation of Microvilli

In an attempt to find a morphological change in the tunicate egg surface associated with fertilization we prepared *Ascidia nigra* eggs for electron microscopy as described elsewhere[1] at different times after insemination, in a range from 30 sec to 20 min.

The unfertilized egg is covered by a smooth plasma membrane[1]. Within 30 sec after insemination, microvilli measuring about 1 μ in length are seen to project from the plasma membrane (Figure). They are present throughout the first 20 min of development. The Table lists the frequencies with which microvilli were observed in this analysis. It is difficult to estimate the size of the area covered by microvilli, but it is probably smaller than about $^1/_3$ of the egg surface. No microvilli were observed in the immediate vicinity of points of sperm-egg contact[2].



Microvilli protruding from the plasma membrane of fertilized *Ascidia nigra* eggs. × 18,200.

Sample	No. of eggs sectioned[a]	No. of eggs showing microvilli
Unfertilized eggs	62	0
Fertilized eggs, fixed between 30 sec and 20 min after insemination	170	70[b]

[a] An average of about 30 sections were examined of each egg.
[b] Individual sections showed between 1 and 50 microvilli, usually in one cluster which never covered more than about $^1/_3$ of the egg's circumference.

Zusammenfassung. Eine elektronenmikroskopische Untersuchung der Eioberfläche von *Ascidia nigra* zeigte, dass 30 sec nach Besamung zahlreiche Mikrovilli in den perivitellinen Raum vorragen. Diese Villi wurden in unbesamten Eiern nie gefunden.

E. Schabtach, L. Stein and H. Ursprung

Department of Biology, The Johns Hopkins University, Baltimore (Maryland 21218, USA), 6 November 1967.

[1] H. Ursprung and E. Schabtach, J. exp. Zool. *156*, 253 (1964).
[2] Work carried out at the Bermuda Biological Station, St. George's West, Bermuda, under an award from the Lalor Foundation. Contribution No. 427 of the Bermuda Biological Station.

A New Aspect of the Anti-Stress Effect of Kinetin

Cytokinins are known to increase plant resistance to diverse stress conditions such as heat or cold treatment[1,2], detachment of leaves[3], effect of metabolic inhibitors[4], parasitic attack[5], salt and water stress[6], effect of herbicides and pesticides[7]. The wide spectrum of the normalizing effects of cytokinins[8] has led Lang to regard them as veritable 'anti-stress' factors[9]. In an extension of this idea, experiments were carried out to show that kinetin is able to counteract metabolic changes associated with mechanical damage to plant tissues.

A characteristic feature of metabolic alterations which follow mechanical injury to leaf tissues is a rapid increase in ribonuclease (RNase) activity[10,11]. The available evidence indicates that DNA-dependent RNA synthesis is involved in this process[11]. The injury-induced increase in RNase activity was chosen as a model system to test the effectiveness of kinetin in counteracting the biochemical alterations evoked by mechanical damage.

Subtle injury and consecutive increase in RNase activity can be induced in tobacco leaf tissues by gentle rubbing of the leaf surface with carborundum or by rapid infiltration of the tissues with distilled water[10,11]. In both cases a rise in RNase activity sets in 1–2 h after damaging the tissues. In the present experiments, the infiltration method was used to induce mechanical damage (stress).

Discs were punched from the leaf tissues of *Nicotiana tabacum* 'White Burley' plants and rapidly infiltrated with water or kinetin solutions of various concentrations. The excess of water was evaporated from the intercellular spaces and the discs were incubated on wet filter paper in Petri dishes. After various incubation periods, the RNase activity was assayed as described earlier[11].

It may be seen from the Table that in the tissues exposed to stress (infiltration of water) the RNase activity markedly increased. Kinetin-treatment reduced the rise in RNase level. Other growth regulators including gibberellic acid or β-indoleacetic acid proved to be ineffective.

[1] L. Engelbrecht and K. Mothes, Flora *154*, 279 (1964).
[2] T. Shirakawa, R. R. Dedolph and D. P. Watson, Proc. Am. Soc. hort. Sci. *85*, 642 (1964).
[3] A. E. Richmond and A. Lang, Science *125*, 650 (1957).
[4] L. Engelbrecht and K. Nogai, Flora *154*, 267 (1964).
[5] L. Lovrekovich and G. L. Farkas, Nature *198*, 710 (1963).
[6] A. Ben-Zioni, C. Itai and Y. Vaadia, Pl. Physiol., Lancaster *42*, 361 (1967).
[7] S. Kuraishi, Lecture at the US-Japan Seminar on Plant Growth Regulators, Kyoto (1966[9]).
[8] D. S. Letham, A. Rev. Pl. Physiol. *18*, 349 (1967).
[9] A. Lang, Science *157*, 589 (1967).
[10] T. O. Diener, Virology *14*, 177 (1961).
[11] G. Bagi and G. L. Farkas, Phytochemistry *6*, 161 (1967).