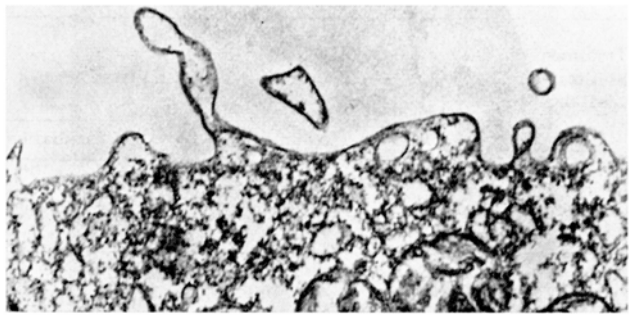
A Fertilization Reaction in *Ascidia nigra*: Formation of Microvilli

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.

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[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.

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Effect of growth regulators on the increase in RNase activity induced by mechanical damage in tobacco leaf tissues

Treatment (infiltrated substance)	RNase activity: Δ O.D.$_{260\,nm}$/100 mg fresh weight/30 min[a]			
	4 h incubation		24 h incubation	
	Experiment No.			
	1[b]	2[b]	3[b]	4[b]
None (0-time control)	29.4	25.1	47.0	28.6
H_2O	61.9	67.8	80.4	33.4
Kinetin $5 \times 10^{-4} M$	44.8	38.4	57.7	22.1
Kinetin $10^{-4} M$	49.9	57.8	67.2	26.9
Kinetin $10^{-5} M$	50.8	58.3	–	–
None (0-time control)	12.5	12.7	34.8	14.6
H_2O	27.0	22.6	67.2	25.9
Gibberellic acid $10^{-4} M$	30.0	23.0	–	–
Gibberellic acid $10^{-5} M$	27.5	25.9	–	28.0
Gibberellic acid $10^{-6} M$	30.6	24.8	66.8	23.5
None (0-time control)	12.7	17.3	17.8	11.6
H_2O	22.2	35.9	47.5	37.8
β-Indoleacetic acid $10^{-4} M$	18.5	37.9	–	–
β-Indoleacetic acid $10^{-5} M$	18.0	38.2	–	–
β-Indoleacetic acid $10^{-6} M$	19.0	39.0	47.3	36.6

[a] Tissue extracts were incubated with yeast RNA. RNase activity is expressed as the increase in absorbance at 260 nm during incubation of the fraction not precipitated with uranyl acetate and trichloroacetic acid (McFadyen reagent). [b] Representative experiments.

The high speed of the kinetin-effect seems to be significant. The inhibition by kinetin of the injury-induced increase in RNase level was apparent in short term experiments (in 3–4 h after treatment). This inhibition seems to be one of the fastest responses of plant tissues to kinetin-treatment described so far.

It is noteworthy that the RNase level of plant tissues is increased not only upon mechanical damage but also under the effect of other stress conditions[12] including leaf excision[13]. Thus the rapid increase in RNase level in leaf tissues seems to be an indication of stress in general. This conclusion is supported by the observation that the increase in RNase activity due to leaf excision is also counteracted by kinetin[13].

Zusammenfassung. Die mechanische Schädigung von Blattgeweben (das Abreiben der Blattoberfläche mit Karborundum oder eine rapide Infiltration der Gewebe mit H_2O) führt zu einer Steigerung der Ribonukleaseaktivität. Die Behandlung der Gewebe mit Kinetin, nicht aber die mit anderen Wachstumsregulatoren antagonisiert die Wirkung der mechanischen Verletzung (Stress) auf die Ribonukleaseaktivität.

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The Localization and Functional Significance of Alkaline Phosphatase in the Vertebrate Ovary

There is considerable confusion about the distribution and functional significance of alkaline phosphatase (AP) in the vertebrate ovary. Most of the previous histochemical work on its localization has been done on the ovaries of a variety of mammalian species[1]; its presence has been generally associated with known active sites of steroidogenesis[2–4]. Very little or no work has been done on the study of the distribution of AP in the ovaries of different groups of vertebrates. The present study was undertaken to determine its functional significance by comparing and contrasting the sites of its distribution in the ovaries of a variety of vertebrates (fishes, *Oxygaster clupeoides* and *Labeo gonius*; amphibian, *Bufo stomaticus*; reptiles, *Hemidactylus flaviviridis, Calotes versicolor, Eryx J. Johnii*; birds, *Passer domesticus, Columba livia*; mammal, white rat). Material was processed by GÖMÖRI's technique for AP[5,6]. Only those components of the ovary were considered to contain AP activity which gave negative reaction in the control sections treated with the buffer without B-glycerophosphate[5,6].

The theca interna of developing follicles of all the species included in this study is strongly AP-positive (Figure 1). The AP activity is absent in the germinal epithelium, follicular epithelium, ooplasm and liquor folliculi of rat ovary. The yolk elements in the developing eggs of submammalian vertebrates give a positive reaction (Figure 1), which continues to persist in the control sections. The AP activity is also very prominent in the theca interna elements of degenerating follicles (Figure 2), which have been considered to constitute the interstitial gland tissue of vertebrate ovary[7–15]. This is in agreement with the previous observations on the mammalian ovary[1,4]. After ovulation, the hypertrophied theca interna of submammalian vertebrate ovary shows abundant AP activity, which is absent in the follicle cells of postovulatory follicles. However, the luteal cells of rat ovary are AP-positive, as also described previously[1].

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