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.

G. BAGI and G. L. FARKAS

Institute of Plant Physiology, Eötvös University, Budapest (Hungary), 22 November 1967.

[12] L. D. DOVE, Pl. Physiol. *42*, 1176 (1967).
[13] J. UDVARDY, G. L. FARKAS, E. MARRÈ and G. FORTI, Physiologia Pl. *20*, 781 (1967).

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].

[1] H. JACOBY, in *The Ovary* (Ed. S. ZUCKERMAN; Academic Press, New York 1962), vol. 1, p. 189.
[2] D. G. MCKAY, J. H. M. PINKERTON, A. T. HERTIG and S. DANZIGER, Obstet. Gynec., N.Y. *18*, 13 (1961).
[3] J. M. CRAIG, Am. J. Obstet. Gynec. *97*, 100 (1967).
[4] E. C. ADAMS, A. T. HERTIG and S. FOSTER, Am. J. Anat. *119*, 303 (1966).
[5] A. G. E. PEARSE, *Histochemistry*, 2nd edn (J. and A. Churchill Ltd., London 1960).
[6] T. BARKA and P. J. ANDERSON, *Histochemistry. Theory, Practice and Bibliography* (Harper and Row Publishers Inc., N.Y. 1963).
[7] S. S. GURAYA, J. Morph. *117*, 151 (1965).
[8] S. S. GURAYA, Am. J. Obstet. Gynec. *96*, 907 (1966).
[9] S. S. GURAYA, Am. J. Obstet. Gynec. *98*, 99 (1967).
[10] S. S. GURAYA, Nature *214*, 614 (1967).
[11] S. S. GURAYA, Acta Anat., in press (1968).
[12] S. S. GURAYA, Acta Anat., in press (1968).
[13] S. S. GURAYA and G. S. GREENWALD, Anat. Rec. *149*, 411 (1964).
[14] S. S. GURAYA and G. S. GREENWALD, Am. J. Anat. *114*, 495 (1964).
[15] S. S. GURAYA and G. S. GREENWALD, Am. J. Anat. *116*, 257 (1965).

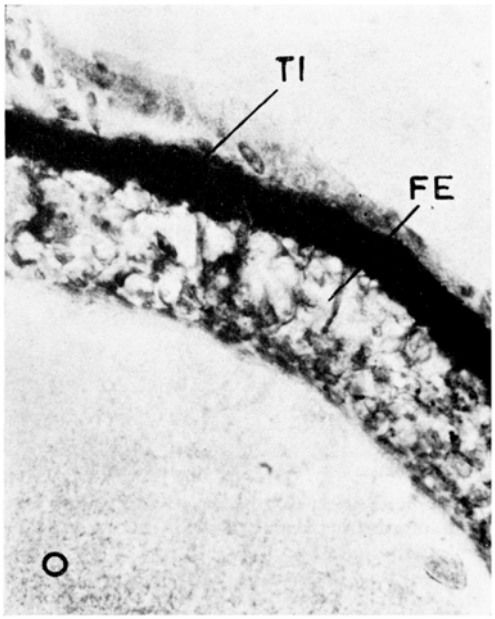

Fig. 1. Photomicrograph of portion of normal developing follicle of *Hemidactylus*, showing strong alkaline phosphatase activity in the theca interna (TI). Note the light positive reaction of the ooplasm (O) and follicular epithelium (FE), which continues to persist in the control sections. × 280.

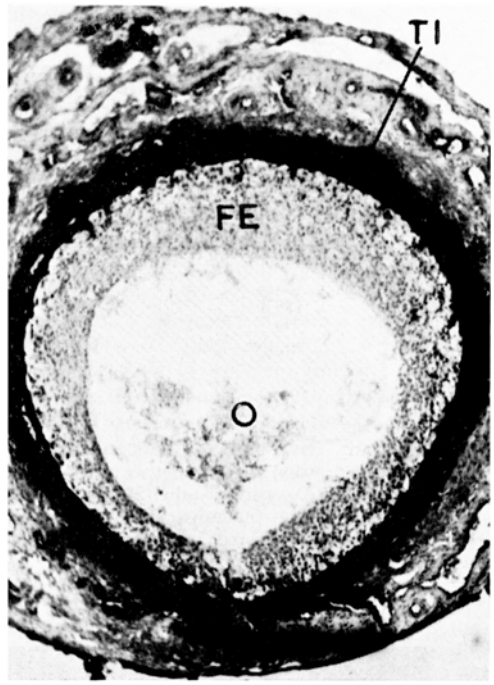

Fig. 2. Degenerating follicle of *Passer*, showing very prominent alkaline phosphatase activity in theca interna (TI). × 70.

The sites of localization of AP activity are highly vascularized, indicating some close physiological relationship between the enzyme and blood vascularity of ovarian compartments. The AP activity present in the theca interna, interstitial gland tissue and corpora lutea must be, therefore, involved in the transference of nutrient and secretory materials or waste products across the cellular membranes of blood vessels and adjacent tissue; it is probably done by the breakdown of phosphate esters. Thus, the AP plays an important role in facilitating the transport of substances across the cellular membranes in the vertebrate ovary. A similar suggestion has also been made by other workers because AP is always found to be associated with the brush borders of the small intestine, kidney tubule cells, and the peripheral portions of nervous elements and a variety of other cells or tissues[5,16–19].

Zusammenfassung. Es zeigt sich, dass die Wirkung der alkalischen Phosphatase in der Theca interna, im interstitiellen glandulären Gewebe und im Corpora lutea liegt und eine bedeutende Transportfunktion bei den ovariellen Zellmembranen der Wirbeltiere hat.

S. K. Varma and S. S. Guraya

Department of Zoology, University of Udaipur, Udaipur (Rajasthan, India), 21 July 1967.

[16] M. S. Burstone, *Enzyme Histochemistry* (Academic Press, New York 1962).
[17] H. B. Tewari and G. H. Bourne, Acta histochem. *13*, 323 (1962).
[18] H. B. Tewari and G. H. Bourne, Acta histochem. *17*, 197 (1964).
[19] Thanks are due to Prof. H. B. Tewari for laboratory facilities.

Relationship Between the Pars Intermedia and the Pars Nervosa in the Hypophysis of an Antarctic Seal

Using light microscopy, fibres coming from the pars nervosa have been described to be present in the pars intermedia of various species. These fibres sometimes have neurosecretory characteristics. In 1949 Bargman[1] working with cats and dogs, found colloidal drops and Herring bodies in some zones of the pars intermedia and suspected them of being of axonal nature. The pars intermedia of fishes seems to be closely related to the pars nervosa, because it has been shown by Scharrer[2] and Meurling[3] that there is an important number of neurosecretory fibres amongt he glandular cells.

Vasomotor fibres in amphibia were described by Green[4]. Although Hild[5] did not find fibres in the pars intermedia of these animals, they were found and identified as neurosecretory by Dawson[6]. In reptiles Hild[5] observed Gömöri positive granules in the pars intermedia near the neurohypophysis.

Using electron microscopy, these findings were confirmed in fishes by Bargman[7]. Knowles[8], also working with fishes, affirms to have distinguished 2 types of fibres in the pars intermedia: one, with elemental granules, similar to those of neurosecretion, and the other contain-