lysine and the common intermediate in the synthesis of a range of cross linking compounds, of which lysinonorleucine is one.

Isotope peak 5 has an elution volume identical to lysinonorleucine and indicates that this compound is participating in the interchain stabilization of this unique α-protein. The elution positions of isotope peaks 3 and 4 closely resemble those of hydroxynorleucine and chloronorleucine, 2 further compounds detectable in proteins cross-linked by lysine derivatives[7]. Peaks 1 and 2 are harder to speculate upon but may be yet further Schiff bases such as dihydroxynorleucine[8] or hydrolysis artefacts[7].

The cross-linking of collagen and elastin has been shown to depend largely on the modification of lysine and hydroxylysine residues in the polypeptide chain. Oxidative 'maturation' reactions give rise to a family of unique cross links, some aldehydic in nature, and all having free amino groups (2). Thus in theory these compounds should be detectable by amino acid analysis and their lack of appearance on a ninhydrin trace is explained by the fact that less than 1 residue of cross link per 1000 amino acid residues is enough to stabilize long stretches of polypeptide, but insufficient to be detected by amino acid analysis.

The capsule protein of *B. undatum* is neither a collagen or an elastin, though it bears resemblances to both. It seems likely however that its interchain cross links may be the same as those found almost exclusively in vertebrate connective tissue and further work is being carried out to confirm this.

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Stimulation of Ribonuclease Activity and its Isoenzymes in Germinating Seeds of Cowpea (*Vigna sinensis*) by Gibberellic Acid and Adenosine-3′,5′-Cyclic Monophosphate

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Summary. Application of GA_3 and cyclic AMP to cowpea seedlings caused a 2–3 fold stimulation of RNAase activity, together with the augmentation of RNAase isoenzymes. Inhibitor studies indicated the requirement of fresh RNA and protein synthesis for enzyme stimulation.

In recent years, controversial views have been expressed concerning the physiological and biochemical role of cyclic AMP in higher plants. Some workers have claimed the natural occurrence of cyclic AMP in plant tissues[1–3], while others were unable to demonstrate its presence[4–6]. Cyclic AMP has been regarded as a mediator of plant growth substances[7–9]. This view has been questioned[10–12], and therefore the precise relationship between plant hormone and cyclic AMP needs further investigation[13,14]. Nevertheless, exogenous application of cyclic AMP is reported to trigger the activity of several hydrolytic enzymes, such as α-amylase[15–17], protease, acid phosphatase[7], ATPase[9], isocitrate lyase[18] and tryptophan oxygenase[19]. In addition, cyclic AMP has also been implicated in RNA synthesis[20,21]. In the present communication, we report the stimulation of RNAase activity and its isoenzymes in seedlings of cowpea by the exogenous application of GA_3 and cyclic AMP.

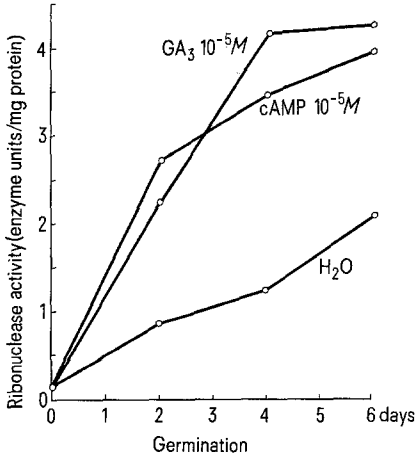

Fig. 1. Time course studies showing the enhancement of RNAase activity in cowpea seedlings in presence of GA_3 and cyclic AMP. The seedlings were grown at 35 ± 2°C in dark.

Materials and methods. The seeds of cowpea (*Vigna sinensis*) were surface sterilized with 0.1% solution of $HgCl_2$ for 5 min and germinated in dark at 35° ± 2°C. Chloramphenicol (20 μg/ml) was added to prevent bacterial contamination. Seedlings (10 g) were homogenized in 20 ml of 0.05 *M* phosphate buffer, pH 6.5 at 4°C. The homogenate was centrifuged in Sorvall at 10,000 *g* for 20 min in cold. The supernatant (crude extract) was employed for measuring RNAase activity, according to the procedure of KALNITSKY et al.[22]. Protein was estimated by the procedure of LOWRY et al.[23]. The crude extract was fractionated on acrylamide gel electrophoresis, using the technique of DAVIS[24]. The method of WILSON[25] was adopted for developing the isoenzymes of RNAase on acrylamide gels.

Results and discussion. Both GA_3 and cyclic AMP promoted 2-3-fold stimulation of RNAase activity in 96 h old seedlings of cowpea (Figure 1). Relatively high concentration of cyclic AMP (10^{-5} *M*) was required to achieve stimulation of RNAase activity comparable to that observed with GA_3, 10^{-7} *M* (Table I). Addition of structural analogues of adenine (e.g., adenosine, AMP, ADP, ATP) showed only 20–70% stimulation of RNAase activity over the controls. These values were relatively less effective than cyclic AMP which gave 165% enhanced enzyme activity (Table II). Seedlings raised in presence of GA_3 10^{-8} *M* + theophylline 10^{-5} *M* showed no additive effect on RNAase activity. Similarly, there was no significant additive increase in RNAase activity at optimum

Table I. Effect of theophylline (TP), GA_3 and cyclic AMP in various combinations on the RNAase activity in the seedlings of cowpea germinated for 96 h in dark at 35 ± 2°C

Additions	RNAase activity (enzyme units/mg protein)	Stimulation over control (%)
Control	1.10	—
TP 10^{-5} *M*	2.18	98
GA_3 10^{-8} *M*	1.73	57
GA_3 10^{-7} *M*	2.60	136
GA_3 10^{-5} *M*	2.68	144
cAMP 10^{-6} *M*	2.16	96
cAMP 10^{-5} *M*	2.55	132
GA_3 10^{-8} *M* + TP 10^{-5} *M*	2.05	87
cAMP 10^{-6} *M* + TP 10^{-5} *M*	2.74	149
cAMP 10^{-5} *M* + TP 10^{-6} *M*	2.30	110
GA_3 10^{-8} *M* + cAMP 10^{-6} *M*	2.34	113
GA_3 10^{-5} *M* + cAMP 10^{-5} *M*	2.42	120

GA_3, cAMP and TP were dissolved in sterile water and were present throughout the period of germination. The seedlings were rinsed with sterile water before homogenization. Enzyme activity was measured in crude extracts.

Table II. Effect of cyclic AMP and analogues of adenine on the RNAase activity in cowpea seedlings after 96 h of germination in dark at 35° ± 2°C

Additions	RNAase activity (enzyme units/mg) protein)	Stimulation over control (%)
Control	1.28	—
cAMP 10^{-5} *M*	3.46	170
cAMP 10^{-4} *M*	3.40	165
Adenine 10^{-4} *M*	1.56	20
Adenosine 10^{-4} *M*	1.85	44
AMP 10^{-4} *M*	2.16	70
ADP 10^{-4} *M*	2.16	70
ATP 10^{-4} *M*	1.95	50

All the substances were dissolved in sterile water and were present throughout the period of germination. The seedlings were rinsed thoroughly with sterile water before processing. Enzyme activity was measured in crude extracts. The values represent an average of 2 experiments.

Table III. Effect of cycloheximide (CHI), actinomycin D (Act D) and abscisic acid (ABA) on GA_3 and cyclic AMP stimulated activity of RNAase in cowpea seedlings (96-h-old) raised in dark at 35 ± 2°C

Additions	RNAase activity (enzyme units/mg protein)	Control (%)
Control	1.01	100
GA_3 10^{-5} *M*	2.88	288
GA_3 10^{-5} *M* + CHI 10 µg/ml	0.46	46
GA_3 10^{-5} *M* + Act D 50 µg/ml	0.44	44
GA_3 10^{-5} *M* + ABA 200 µg/ml	0.90	90
cAMP 10^{-5} *M*	2.67	267
cAMP 10^{-5} *M* + CHI 10 µg/ml	0.45	45
cAMP 10^{-5} *M* + Act D 50 µg/ml	0.43	43
cAMP 10^{-5} *M* + ABA 200 µg/ml	0.89	89

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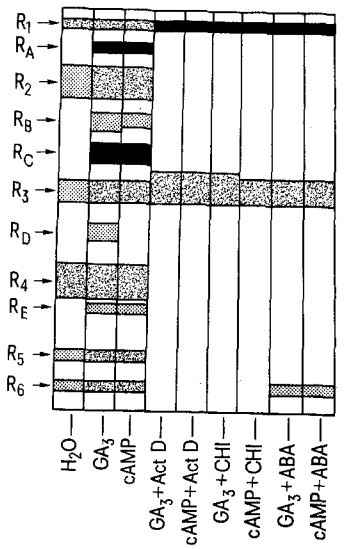

Fig. 2. Diagrammatic representation of RNAase isoenzymes on acrylamide gels. Crude extracts prepared from seedlings raised in presence of H_2O, GA_3 10^{-5} *M*, cAMP 10^{-5} *M*, GA_3 10^{-5} *M* + Act D 50 µg/ml, cAMP 10^{-5} *M* + Act D 50 µg/ml, GA_3 10^{-5} *M* + CHI 10 µg/ml, cAMP 10^{-5} *M* + CHI 10 µg/ml, GA_3 10^{-5} *M* + ABA 200 µg/ml and cAMP 10^{-5} *M* + ABA 200 µg/ml were fractionated on acrylamide gels. The seeds were germinated in dark for 96 h at 35 ± 2°C. GA_3 and cAMP were dissolved in sterile water. ■, High activity; ▨, medium activity; ▩, low activity.

concentrations of GA_3 10^{-5} M + cyclic AMP 10^{-5} M, or cyclic AMP 10^{-5} M + theophylline 10^{-5} M. However, slight additive effect was observed at suboptimal concentrations of GA_3 10^{-8} M + cyclic AMP 10^{-6} M, or cyclic AMP 10^{-6} M + theophylline 10^{-5} M. Application of theophylline alone showed nearly 2-fold stimulation of RNAase activity (Table I). These results corroborate the view that GA_3 and cyclic AMP stimulate RNAase activity by acting at a common site. The stimulation of RNAase activity elicited by GA_3 and cyclic AMP was extremely sensitive to the action of Act D and cycloheximide. Both antibiotics inhibited the enhanced RNAase activity to the extent of about 80% (Table III). Thus, RNA and protein synthesis seem necessary for the GA_3 and cyclic AMP promoted activity of RNAase. This was further supported by the fact that both GA_3 and cyclic AMP enhanced the incorporation of ^{3}H-uracil and ^{3}H-leucine into RNA and protein fractions respectively, which was strongly inhibited by Act D and CHI (Tables IV and V). Abscisic acid also suppressed the enhanced RNAase activity evoked by GA_3 and cyclic nucleotide (Table III).

Table IV. Effect of GA_3 and cyclic AMP on ^{3}H-uracil incorporation into RNA fraction

Additions	^{3}H-uracil incorporation	
	cpm/mg protein	Control (%)
Control	1057	100
Act D 50 µg/ml	251	24
GA_3 10^{-5} M	1867	177
GA_3 10^{-5} M + Act D 50 µg/ml	400	38
cAMP 10^{-5} M	1530	145
cAMP 10^{-5} M + Act D 50 µg/ml	350	34

The seeds were germinated for 96 h at 35 ± 2 °C. ^{3}H-Uracil (1 µCi/ml, Spec. act. 6100 mCi/mmole) was added after 48 h germination of seeds. GA_3, cAMP and Act D were added at the beginning of germination.

Table V. Effect of GA_3 and cyclic AMP on ^{3}H-leucine incorporation into protein fraction

Additions	^{3}H-L-leucine incorporation	
	cpm/mg protein	Control (%)
Control	1628	100
CHI 10 µg/ml	265	16
GA_3 10^{-5} M	2667	164
GA_3 10^{-5} M + CHI 10 µg/ml	338	21
cAMP 10^{-5} M	2583	159
cAMP 10^{-5} M + CHI 10 µg/ml	355	22

The seeds were germinated for 96 h at 35 ± 2 °C. ^{3}H-L-leucine (2 µCi/ml Spec. act. 7600 mCi/mmole) was added after 48 h germination of seeds. GA_3, cAMP and cycloheximide (CHI) were added at the beginning of germination.

Fractionation of crude extracts prepared from 96 h old seedlings revealed six isoenzyme bands (R_1–R_6) on acrylamide gels. The stimulatory effect of GA_3 and cyclic AMP on enzyme activity was accompanied by the quantitative and qualitative changes in the isoenzyme pattern of RNAase. Several RNAase isoenzyme bands eluted from the acrylamide gels, revealed enhanced activity in GA_3- and cAMP-treated seedlings (unpublished results). Out of 6 isoenzymes observed in controls, there was a distinct augmentation in the intensity of 4 isoenzyme bands (R_2, R_3, R_5, R_6) by the application of GA_3 and cyclic AMP. In addition, the hormone (GA_3 10^{-5} M) treatment resulted in the appearance of 5 new minor bands (R_A, R_B, R_C, R_D, R_E). The action of GA_3 was mimicked by cyclic AMP (10^{-5} M), since it also caused the appearance of 4 identical minor bands in addition to the augmentation of the pre-existing activity bands (R_A, R_B, R_C, R_E) (Figure 2). These studies suggested a similar response of RNAase isoenzymes to GA_3 and cyclic AMP. Addition of Act D along with GA_3 or cyclic AMP completely abolished the appearance of all the newly formed minor activity bands. Thus, only 2 RNAase isoenzymes (R_1, R_3) were observed when Act D was added to GA_3- and cyclic AMP-treated seedlings. Cycloheximide caused an inhibitory response similar to that observed with Act D. Addition of ABA to seedlings raised on GA_3 or cyclic AMP medium abolished the appearance of all the newly formed isoenzymes and also the 3 pre-existing activity bands (R_2, R_4, R_5). The action of ABA resembled the inhibitory response of Act D and CHI, except that the R_6 band persisted in addition to R_1 and R_3 (Figure 2). The modulation of RNAase isoenzymes by GA_3 and cyclic AMP in germinating cowpea seeds could be at the transcriptional and translational levels. However, the possibility of activation, inhibition or aggregation cannot be ruled out unequivocally unless the de novo synthesis of RNAase is established in this system.

Zeitgeber Induced Modulation of Activity Patterns in Nocturnal Mammals (Chiroptera)[1]

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Summary. Changing the L:D intensity ratio of a synchronizing light-dark regime leads to characteristic modulations of the activity pattern of 5 Chiroptera species. These modulations fit the predictions of WEVER's oscillator model.

WEVER's oscillator model[2–5] makes the most explicit predictions about the characteristics of circadian systems under different Zeitgeber conditions, of all hitherto developed models of biological 24-hour-periodicity (for review see[6]). According to one of these predictions, increasing L:D intensity ratio and decreasing mean intensity of illumination of a synchronizing light-dark-

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