TABLE I

Growth response of the diauxotrophic mutant of T. bovina *on hypertonic media at three levels of temperature*

Incubation temperature**	KCl in minimal medium						Sucrose in minimal medium			
	0·0 molal		0 067 molal		1·33 molal		0·0 Molal		0·067 Molal	
	Mutant	Wild*	Mutant	Wild	Mutant	Wild	Mutant	Wild	Mutant	Wild
27° C	−	+	±	+	−	+	−	+	±	+
32° C	−	±	+	±	+	±	−	±	+	±
37° C	−	−	+	−	+	−	−	−	+	−

Note.—The mutant (ad⁻, meth⁻) colonies grown on minimal medium (supplemented with adenine and methionine) were replica-plated[6] on minimal medium agar plates (supplemented with methionine only) containing either potassium chloride or sucrose. Growth was observed for over a period of 3 days.

* Wild-type was also replica-plated as control.

** Normal temperature for growth is 26–27° C. The wild-type and the mutant growth poorly at 32° C and do not grow at 37° C.

+ = Normal growth; ± = poor growth; − = no growth.

lacking adenine and made hypertonic are seen in Table I.

From the results it can be seen that the mutant colonies did not grow on media not supplemented with either potassium chloride or sucrose incubated at the above temperatures, suggesting that the induced mutation was not temperature sensitive. However, the mutant showed poor to no growth either on potassium chloride or sucrose supplemented media incubated at 27° C. On the other hand, there was efficient growth of the mutant on hypertonic media incubated at 32° and 37° C, indicating that the mutation induced in the adenine biosynthetic pathway was temperature dependent osmotic remedial. Mutants exhibiting growth on minimal medium in response to temperature changes or increased osmotic pressure or combination of both, have been interpreted as due to missense mutations[3–5]. Missense mutations induced in DNA lead to the substitution of one amino acid for another in the polypeptide chain. Such a change may result in a partial enzymatic inactivation which becomes absolute under specific conditions of temperature and pH. Mutations of simulai kind might be involved in the mutant of *T. bovina* in the adenine biosynthetic pathway enabling the growth on hypertonic medium at higher temperatures.

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May 18, 1978.

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CYTOCHEMICAL STUDIES ON THE EFFECT OF KINETIN ON CUCUMBER COTYLEDONS

KINETIN-inducible developmental steps depend upon RNA synthesis[1]. An open question is how kinetin stimulates RNA synthesis. There are several pathways for its mode of action[2]. The present study examines a novel effect of kinetin on histone composition and subsequent changes in endogenous levels of RNA in isolated cucumber cotyledons.

Seeds of *Cucumis sativus* were surface sterilized with 0·01% mercuric chloride and germinated in dark at 25 ± 2° C. Cotyledons were excised and floated on distilled water or on 10 ppm kinetin since it showed maximum stimulatory effect on the expansion growth of cotyledons (Table I). After 24 and 48 hours of incubation cotyledons were fixed in Carnoy's fluid and neutral buffered formalin. In 10 microns thick sections of cotyledons, RNA and histone fractions were localized in the manner suggested by Tepper and Gifford[3] and Black and Ansley[4] respectively. Extinction values and relative content of RNA were measured with the help of cytophotometer[5,6].

TABLE I

Effect of different concentration of kinetin on cucumber cotyledon expansion
The values represent mean of thirty replicates

Period in hours	Cotyledon area in mm²					
	Control	Kinetin (concentration in ppm)				
		0·01	0·1	1·0	10·0	20·0
0	47·0	47·0	47·0	47·0	47·0	47·0
24	49·25	57·0	57·0	72·0	81·2	85·2
48	57·25	69·70	71·5	98·0	130·7	125·0
72	70·76	83·2	85·4	113·4	157·0	138·1
96	77·80	83·0	85·8	120·0	178·0	170·0

TABLE II

Effect of kinetin on endogenous RNA level, cell area and nuclear volume.
The data represent the average of fifty replicates

Period in hours	RNA content/cell in arbitrary unit		Cell area (μ^2)		Nuclear volume (μ^3)	
	Control	Kinetin	Control	Kinetin	Control	Kinetin
0	6·012	6·012	89·0	89·0	6·37	6·37
24	6·662	18·04	108·5	198·0	11·49	82·49
48	6·880	31·20	117·0	262·5	29·66	84·33

Changes in the content of RNA in cells of cotyledon after kinetin application are shown in Table II. Initially the cells contain about 6·0 arbitrary units of RNA. In control no change in RNA content is observed upto 48 hours of incubation. On the other hand a pronounced increase is observed in the endogenous level of RNA after kinetin application. The 24 hour cells of kinetin-treated cotyledons contain more than two times the RNA of the control. This difference becomes five-fold in 48 hours. A similar trend is also observed in the size of cotyledons and their cells. Kinetin (10 ppm) brings about two-fold increase in the area of cotyledons as well as their cells (Tables I, II). Rijven[7] showed that light-promoted cell expansion of detached fenugreek cotyledons was mimicked by kinetin application, and that the expansion of cotyledons and net RNA synthesis were inhibited by actinomycin-D, 6-methyl purine and thiouracil. We also observed a fair correlation between the increase in endogenous RNA level and the expansion of the cells in kinetin treated cotyledons. After kinetin application, a marked increase in nuclear volume was observed. At 24 hours of incubation, nuclei of kinetin treated cotyledons showed seven-fold increase in their size over the control which was reduced to about 3 times after 48 hours of incubation (Table II).

An interesting point is the effect of kinetin on the composition of histones. Ammoniacal silver nitrate reaction detects two fractions of histones, *viz.*, arginine rich and lysine rich. Black granules in the microphotograph (Fig. 1) indicate the presence of arginine fraction and the faint background in the nucleus (actually brown colour in the stained preparation) represents lysine rich histones. Nuclei of control cotyledons are rich in lysine rich histones (Fig. 1 A, C) while kinetin-treated ones contain more of arginine rich histones (Fig. 1 B, D). Sauter[8] concluded that RNA synthesis proceeds mainly in diffuse chromatin, the dense chromatin being inactive with respect to DNA directed RNA synthesis. Further he emphasized that lysine rich histones cross linking the chromatin fibrils are responsible more for the formation

of dense inactive chromatin as compzred to arginine rich ones. We found that kinetin alters the composition of histones from lysine to arginine, which may be the probable cause for the increase in the endogenous RNA level. A simular alteration in histone composition was observed by Piesco and Alvarez[9] in the nuclei of pea root after kinetin application. Thus the altered composition of specific histones may represent the initial step in preparing the chromatin for high transcriptional activity.

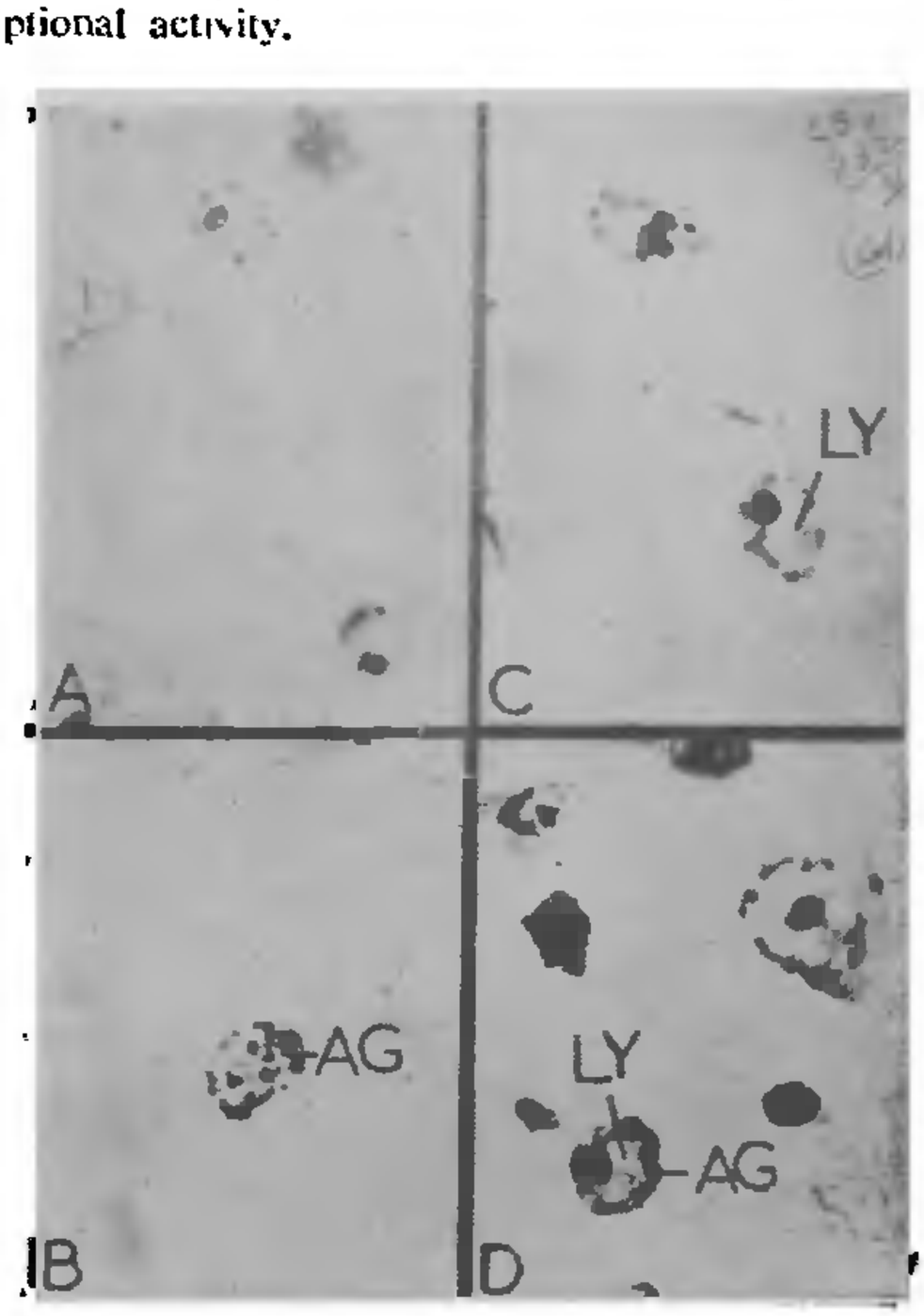

FIG. 1. A–D. Microphotographs of nuclei of control and kinetin treated cotyledons (× 800). Note higher content of lysine rich histones (Ly) in nuclei of control cotyledons incubated for 24 hours (A) and 48 hours (C). On the other hand nuclei of kinetin treated cotyledons show predominant localization of arginine rich histones (AG) at 24 hours (B) and 48 hours (D) of incubation.

The research awards from ICAR and CSIR to KRP is gratefully acknowledged.

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UTILITY OF UNMATED FEMALES OF *PERISIEROLA NEPHANTIDIS* MUESEBECK IN THE BIOLOGICAL CONTROL OF *NEPHANTIS SERINOPA* MEYRICK

Nephantis serinopa Meyrick is one of the serious pests of coconut[2] and is parasitized by a number of native parasites in the field[3,4]. *Perisierola nephantidis* Muesebeck was reported to exert check on the populations of Nephantis larvae[1]. Attempts are made to use this parasite in the control of *N. serinopa*[5] since control by conventional methods is unsatisfactory Mass production of *P. nephantidis* in the laboratory for inundative releases sometimes results in the production of females only. Studies were made on the utility of unmated females of *P. nephantidis* in the biological control of *N. serinopa* and the results are presented in this paper.

A culture of *P. nephantidis* was maintained, at room temperature, on the larvae of *N. serinopa*. The pupae were individually kept in glass tubes for emergence of adults. On emergence the virgin females were confined individually with larvae of *N. serinopa* and fed with honey drops on waxed paper. Similarly females with males at 1 : 1 ratio were confined with host larvae till all the females died. Observations on host mortality sex-ratio of the parasite and progeny production were recorded.

The results revealed that unmated females of *P. nephantidis* paralysed the host laravae as effectively as mated females and laid eggs on them. Mortality of host caused by mated and unmated females was 100%. The progenies produced by mated and unmated females differed widely (Table I). Unmated females produced only males as against both sexes produced by mated females. Both mated and unmated females had three reproductive cycles in their life-time, spread over 12 to 13 days, with four or five days intervals between cycles.