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ROBERT J. WEAVER

Department of Viticulture and Enology,  
University of California,  
Davis, California.  
Dec. 18.

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### Effects of Kinetin on Cell Division in *Paramecium caudatum*

KINETIN (6-furfurylaminopurine) has been reported to stimulate cell division in excised tobacco pith tissues<sup>1</sup>, in onion root tips<sup>2</sup> and, most recently, in sarcoma tumour cells<sup>3</sup>. Lettré and Endo<sup>4</sup> found no changes in cell division or tumour growth with kinetin in cultures of normal or malignant human and animal cells. They concluded that these tissues either did not possess a kinetin-like trigger for cell division, or contained optimal levels of such a substance.

This communication presents some of the findings on changes in rates of division induced by kinetin in a free-living organism, *Paramecium caudatum*.

Stock cultures of *P. caudatum* (obtained from the Hebrew University Department of Zoology) were maintained as clones on hay infusion medium at 25° C. in the dark. Families were begun in depression slides containing a single animal in two drops of hay infusion plus four drops of sterilized tap water. Treatment slides contained hay infusion with kinetin (kindly supplied by Prof. F. Skoog), dissolved in sterilized tap water and added to the hay infusion at the time of treatment. All slides—treatments and controls—had equal proportions of infusion and water and were adjusted to pH 6.5–6.8.

The findings reported here were obtained from tests with isolation cultures using the following procedure. A single individual was isolated in a depression slide. After two divisions, the four resulting animals were transferred each to a new slide—one control and three different concentrations of kinetin solution. Thus each experimental observation was matched by its own control, originated from the same individual. The number of divisions per slide was recorded every 24 hr., after which two animals were transferred from each slide to new medium.

Table 1. COMPARISONS OF DIVISION-RATES OF *Paramecium caudatum* IN CONTROL MEDIUM AND IN VARIOUS CONCENTRATIONS OF KINETIN

Concentration of kinetin (mgm./l.)	No. of 24-hr. period comparisons	Mean No. of divisions/24 hr.		Proportion $\frac{\bar{d}}{\bar{c}}$	$t\bar{c} - \bar{d}$
		Control $\bar{c}$	Kinetin $\bar{d}$		
0.25	60	1.32	1.48	1.12	-1.57
0.5	18	1.24	1.72	1.39	-2.71*
1.0	24	1.42	2.06	1.45	-3.20†
1.5	18	1.24	2.20	1.77	-3.88†
2.0	24	1.42	1.99	1.40	-2.29*
2.5	18	1.24	1.91	1.53	-2.41*
3.0	24	1.42	1.86	1.31	-2.35*
4.0	21	2.36	2.50	1.06	-0.42
10.0	53	1.60	1.25	0.78	3.34†
17.0	35	2.14	0.94	0.44	6.53†
33.0	30	2.08	0.89	0.44	6.30†

\* Significant at 0.05 level. † Significant at 0.01 level.

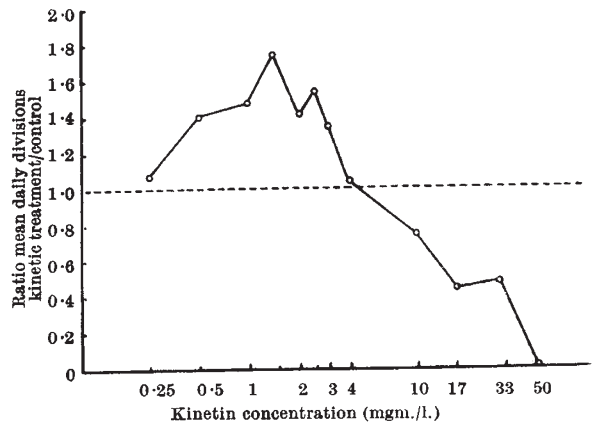


Fig. 1. Variations in division-rate of *P. caudatum* with changing kinetin concentration

Readings were made for three days in succession: at the end of these the experiment was discontinued and a new set begun. Mean division-rates of treatments and controls were compared and their differences tested for significance by Student's *t* test for the mean of differences.

In order to test a possible effect of the kinetin on the bacterial populations of the culture media during the 24-hr. periods, hay infusion samples—with and without kinetin—were tested on agar and in bouillon. No gross changes were observed in either size or constitution of the bacterial populations.

Table 1 shows appreciable changes in mean daily division frequencies (for 3-day periods) in cultures containing a variety of concentrations of kinetin. 100 mgm./l. kinetin caused death of all *Paramecia* within  $\frac{1}{2}$  hr., while 50 mgm./l. blocked division completely without being lethal. (The animal remained alive during the 3-day period but did not divide.) Between 33 mgm./l. and 0.25 mgm./l. we found a sensitive range of responses from significant elevations to severe inhibition of cell multiplication (Fig. 1). As the dose of kinetin decreased from 33 to 4 mgm./l., the ratio of experimental to control divisions rose from 0.44 to approximately 1. The ratio rose steadily above 1 as the concentration dropped from 4 to 1.5 mgm./l., and fell steadily back to 1 as the concentration dropped from 1.5 to 0.25. It is interesting to note that the concentrations found to be most active in our material, as well as in onion and tumour cells<sup>2,3</sup>, have all been of the same order of magnitude.

Tests are under way to determine the time of onset of the effect of kinetin during the life-cycle of the protozoan cell.

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RUTH GUTTMAN  
AVIGDOR BACK

Department of Experimental Medicine  
and Cancer Research,  
Hebrew University—Hadassah Medical School,  
Jerusalem, Israel.  
Dec. 23.

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