Proceedings of the Iowa Academy of Science

Volume 67 | Annual Issue Article 63

1960

The Effect of Kinetin on the Rate of Multiplication of Paramecia

Mary Annunciata McManus
Mount Mercy College

Kathleen Sullivan
Mount Mercy College

The Effect of Kinetin on the Rate of Multiplication of Paramecia

SISTER MARY ANNUNCIATA MCMANUS, R.S.M., AND KATHLEEN SULLIVAN[1]

Abstract. A stimulation of the rate of division of *Paramecium caudatum* by kinetin has been reported. This paper reports investigations in which kinetin was found to be inhibitory, rather than stimulatory, to division of paramecia under similar conditions and at the same concentrations. Addition of IAA apparently counteracted the inhibition produced by kinetin.

Very early in the history of plant physiology, Hans Sachs spoke of "specific substances" in the plant body which might control the formation of plant organs. He postulated a series of separate, specific "factors" which would regulate, in turn, each successive phase of development. From this time, 1880, onward until now, a definite search has been made for such substances, and through the efforts of a long line of workers beginning with Darwin, we now have a great fund of information on the plant hormones, specific chemical substances which regulate plant growth. Kinetin (6-furfurylaminopurine) is one of the most recently discovered of these substances.

In both plants and animals, growth is the result of two activities —cell division and cell enlargement. Much effort has been directed to detecting distinct substances which separately control these two processes.

As is well known, cell enlargement in plants is regulated by indoleacetic acid, a hormonal substance which occurs naturally in plants, and which has as its characteristic effect the production of cell enlargement in the stem. Another class of substances with hormonal activity is the gibberellins, which also are believed to produce cell enlargement.

In 1956, kinetin was discovered. It was the first isolated chemical substance which had been shown to have the ability to regulate the process of cell division. Naturally, it created much excitement.

Kinetin was isolated from DNA by Miller *et al.* (1955) and was later (1956) characterized chemically and synthesized by them at the University of Wisconsin. Since it has become available, kinetin has been the subject of great interest and intensive investigation. Miller and his co-workers found that in combination with indoleacetic acid (IAA), kinetin (K) could cause the proliferation

[1]Mount Mercy College, Cedar Rapids, Iowa.

of parenchyma cells from tobacco pith which were growing in culture. This same stimulatory activity has been tested for on a variety of other plant materials. In a number of such investigations (e.g., Miller, 1956; Dmochowski *et al.*, 1957; Richmond and Lang, 1957; Provasoli, 1958; Pilet *et al.*, 1960) it was found to be stimulatory, but in other cases (e.g., DeRopp, 1956; Kemp, 1957; Lansford *et al.*, 1958; Pilet *et al.*, 1960) an inhibitory effect followed its application.

In 1956, Guttman studied the effect of K on intact roots of *Allium cepa*. By counting mitotic figures in random fields of squash preparations, she concluded that K increases the rate of mitosis in the cells of growing onion root tips. A similar study by McManus (1960) produced data which indicated that K inhibits rather than stimulates mitosis in the cells of the intact onion root tip. In this second study the mitotic figures were counted in serial sections of the root tips. Quantitative chemical studies of the DNA content of cells of the onion root tip treated with K in the same way, made by Jensen and Pollock (1958), also indicate that mitosis is inhibited rather than stimulated under these conditions.

A few studies of the effect of K on animal cells have been made, but with conflicting results (Swann, 1958). Supniewski *et al.* (1957) reported a stimulatory effect on the growth of white mice, *Rana* tadpoles, and *Euglena gracilis*. But Ham (1956) found an inhibitory effect of K in studies of regeneration of hydra tentacles.

Guttman and Back (1958) made a study of the effect of K on the rate of division in *Paramecium caudatum* and reported stimulation. In the present study experiments as described in the publication of Guttman and Back were repeated, using the concentrations which they found stimulatory at a significant level of confidence, and using the technique which they followed. The results here indicate inhibition rather than stimulation.

Materials and Methods

Preliminary experiments were done to determine the best nutrient for growing the *Paramecium caudatum* clone, and a decoction prepared from water containing masses of snail eggs was decided upon as optimum. This was filtered and adjusted to pH 6.8 and used as the nutrient medium.

A stock clone of paramecia was developed from one individual and maintained in the dark on the snail egg extract at 25° C ± 2°. A family was begun by isolating one individual from the stock clone and placing it in a depression slide containing two drops of nutrient and two drops of distilled water. After the isolated paramecium had divided twice, the four resulting paramecia were isolated singly

on depression slides. One was used as a control and the other three were each placed in a different concentration of the kinetin solution. Thus, each paramecium in K was matched by its own control, which had originated from the same individual.

The number of divisions which had occurred on each slide was recorded every 24 hours. If there was one division or more, each resulting individual was transferred singly to a new depression slide in fresh medium of the same concentration. If the paramecium had not divided in 24 hours, its medium was replaced by fresh medium of the same concentration. Each test was continued for three days, the number of divisions being recorded every 24 hours; the test was then discontinued and a new one begun.

Concentrations of K used in the experiment were 0.5 ppm, 1.0 ppm, and 1.5 ppm (the concentrations found stimulatory by Guttman and Back). The control paramecium was placed in a depression containing two drops of nutrient plus two drops of distilled water. Individuals for treatment were placed in a depression containing two drops of nutrient plus two drops of K solution which had been made up at a concentration of either 1, 2, or 3 ppm, so that the final concentration in the depression was either 0.5, 1.0, or 1.5 ppm K.

As it became apparent from the data that K was causing a decrease in the rate of division, a second series of experiments was begun in which K was used in combination with IAA. After preliminary testing, 2 ppm IAA was determined upon as being most stimulatory to cell division. Nutrient was then combined in equal proportions with stock solutions of IAA and K, made up so that final concentrations as listed in Table 2 would result after combination of the three. Excepting for the addition of IAA, this experiment was conducted according to the procedure described for the first series.

Results

Experimental Series 1. The results of the first series of experiments are shown in Table 1, which gives the mean numbers of divisions per 24 hour period for paramecia treated with K as compared with untreated controls.

Table 1
Mean Numbers of Divisions Per 24 Hour Period in Paramecia Treated With Kinetin As Compared With Untreated Controls

Number of 24 hr. comparisons	Control	s	Concentration of K in ppm			t value	Level of significance
			0.5	1.0	1.5		
64	0.843	0.47	0.742			1.7	0.1
60	0.850	0.51		0.650		2.99	0.1
61	0.819	0.49			0.606	3.31	0.01

Sixty-four comparisons were made between paramecia treated with 0.5 ppm K and controls which had descended from the same individual. The mean number of divisions in controls was 0.843, and of those treated with 0.5 ppm, 0.742. The difference between these means was tested with Student's *t* test, and found to be significant at .10 level.

Sixty comparisons were made between paramecia treated with 1.0 ppm K and controls. The mean number of divisions for controls was 0.850 and for the treated paramecia, 0.650. The *t* value here was 2.99, which is significant at a .01 level.

Sixty-one comparisons were made between paramecia treated with 1.5 ppm K and controls. The mean for the controls was 0.819 and for the treated paramecia, 0.606, which gave a *t* value of 3.31, significant at a .01 level.

These data indicate a significant decrease in the number of divisions per 24 hours in paramecia treated with K, the effect increasing with increased concentration of K.

Experimental Series 2. The results of the second series of experiments are given in Table 2, which gives mean numbers of divisions per 24 hour period for paramecia treated with K + 2 ppm IAA as compared with controls.

Table 2

Mean Numbers of Divisions per 24 Hour Period in Paramecia Treated With Kinetin Plus 2 ppm Indoleacetic Acid As Compared With Untreated Controls

Number of 24 hr. comparisons	Control	s	Concentration of K in ppm plus 2 ppm IAA			t value	Level of significance
			0.5	1.0	1.5		
41	0.607	0.48	0.607				
42	0.619	0.48		0.548		0.939	0.35
41	0.610	0.23			0.512	1.271	0.25

Forty-one comparisons were made between paramecia in 0.5 ppm K + 2 ppm IAA and controls. The mean number of divisions for the treated paramecia was identical with the mean for the controls.

Forty-two comparisons were made between paramecia treated with 1.0 ppm K + 2 ppm IAA and control paramecia. The mean number of divisions for the controls was 0.619 and the mean for the treated paramecia, 0.548. A *t* test of the difference shows that it is significant only at a .35 level.

Forty-one comparisons between paramecia treated with 1.5 ppm K + 2 ppm IAA showed a mean of 0.610 for the controls and a mean of 0.512 for the treated paramecia. The difference was significant only at a .25 level.

These data indicate that IAA reversed the inhibition of division produced by 0.5 ppm K, and counteracted the severity of the inhibition produced by 1.0 and 1.5 ppm K.

Discussion

Four classes of chemical substances are now believed to be regulatory in the growth of plant tissues: auxins, of which IAA is the type; gibberellins; long-chain fatty alcohols; and kinins. Yeast extract (Muir *et al.*, 1958) and coconut milk (Steward *et al.*, 1958) are also known to contain regulatory substances which have not yet been isolated. Little is known about the metabolism of these substances in the plant body, and even less about whether they exist and function in animal tissues. The only links connecting them with regulation in animals, which thus far have been established, are through serotonin (Page, 1958), a substance related to mental illness and through which some tranquilizers are thought to have their effect, and 5-hydroxylindoleacetic acid (Schneckloth *et al.*, 1957), which is found in large amounts in the urine of patients with malignant carcinoid tumors.

Kinetin is known to be able to cause division in certain plant cells which would not otherwise divide. But cases have also been found in which the rate of cell division is decreased after its application. Whether it has stimulatory or inhibitory effects on animal tissue, and whether it occurs normally in the animal body and has a regulatory function there, are obviously questions of great interest.

In the series of experiments reported here, an inhibitory effect upon the division of *Paramecium caudatum* was noted when kinetin was used in the same concentrations and under the same conditions as described by Guttman and Back (1958) in a study in which they report stimulation of division by kinetin. The discrepancy might be due to a difference in strain of the animals used.

Additional studies in which K was used in combination with 2 ppm IAA have shown that IAA reverses the inhibitory effect of K at 0.5 ppm, so that treated paramecia divide at the same rate as untreated controls. It counteracted to a lesser degree the inhibition caused by K at higher concentrations, but the magnitude of this effect decreased with increasing K concentration.

There are other reports of antagonism between K and IAA (e.g., Wickson and Thimann, 1957) and of interactions of various kinds between IAA and K and the gibberellins. Interactions between these chemical substances, and between them and other substances and events within the cell which compel its division, must be very complex (Stern, 1956). Their exact nature remains to be determined.

Literature Cited

DeRopp, R. S. 1956. Kinetin and auxin activity. Pl. Physiol. 31 (3): 253-254.
Dmochowski, A., Maciejewska-Potapczykowa, W., and Sempinska. E. 1957. The effect of kinetin isolated from thymus DNA on plant tissues. Acta Soc. Bot. Polon. 26 (2): 361-371.
Guttman, R. 1956. Effects of kinetin on cell division, with special reference to initiation and duration of mitosis. Chromosoma 8: 341-350.
Guttman, R., and Back, A. 1958. Effects of kinetin on cell division in *Paramecium caudatum*. Nature 181: 852.
Ham, R. G., Eakin. R. E., Skinner, C. G., and Shive, W. 1956. Inhibition of regeneration in hydra by certain new 6-(phenylalkyl) aminopurines. J. Am. Chem. Soc. 78: 2648.
Jensen, W. A., and Pollock, E. G. 1958. Effect of kinetin on the protein and nucleic acid content of root tip cells. Pl. Physiol. (Sup.) 33: xv.
Kemp, H. T., Fuller, R. G., and Davidson, R. S. 1957. Inhibition of plant growth by root-drench application of kinetin. Science 126 (3284): 1182.
Lansford, E. M., Jr., Skinner, C. G., and Shive, W. 1958. Augmented inhibition by kinetin and related compounds of growth of pteridine-inhibited *Lactobacillus drabinosus*. Arch. Biochem. and Biophys. 73 (1): 191-197.
McManus. Sister M. A. 1960. Certain mitotic effects of kinetin, gibberellic acid, indoleacetic acid, and maleic hydrazide on the root of *Allium cepa*. Nature 185 (4705): 44-45.
Miller, C. O. 1956. Similarity of some kinetin and red light effects. Pl. Physiol. 31 (4): 318-319.
Miller, C. O., Skoog, F., Okumura, F. Von Saltza, M., and Strong, F. 1955. Structure and synthesis of kinetin. Jour. Amer. Chem. Soc. 77: 2662.
Miller. C. O., Skoog, F., Okumura, F., Von Saltza, M., and Strong, F. 1956. Isolation, structure and synthesis of kinetin, a substance promoting cell division. Jour. Amer. Chem. Soc. 78: 1375.
Muir. W. H., Hildebrandt, A. C., and Riker, A. J. 1958. The preparation, isolation and growth in culture of single cells from higher plants. Amer. Jour. Bot. 45: 589-597.
Page, I. H. 1958. Serotonin (5-hydroxytryptamine); the last four years. Physiol. Reviews 38 (2): 277-335.
Pilet, F., Hildebrandt, A. C., Riker, A. J., and Skoog, F. 1960. Growth in vitro of tissues isolated from normal stems and insect galls. Amer. Jour. Bot. 47: 186-195.
Provasoli, L. 1958. Effect of plant hormones on *Ulva*. Bio. Bull. 114 (3): 375-384.
Richmond, A. E., and Lang, A. 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science 125 (3249): 650-651.
Schneckloth, R., Page, I. H., del Greck, F., and Corcoran, A. C.. 1957. Effects of Serotonin antagonists in normal subjects and patients with carcinoid tumors. Circulation 16 (4): 523-532.
Stern, H. 1956. The physiology of cell division. Amer. Rev. Pl. Physiol. 7: 91-114.
Steward, F. C., Mapes, Marion O., and Smith, Joan. 1958. Growth and organized development of cultured cells. I. Growth and division of freely suspended cells. Amer. Jour. Bot. 45: 693-703.
Supniewski, J., Krupinska, J., Supniewski, H., and Waclaw, B. 1957. The biological properties of kinetine and thiokinetine. Bull. Acad. Polonaise Sci. Ser. Sci. Biol. 5 (1): 19-24.
Swann, M. M. 1958. The control of cell division: a review. II. Special mechanisms. Cancer Research 18 (10): 1118-1160.
Wickson, M., and Thimann, K. V. 1956. The antagonism between kinetin and indolacetic acid in lateral bud development. Pl. Physiol. (Sup.) 31 (28): xxviii.