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The Effect of Kinetin on the Multiplication Rate of *Blepharisma Undulans*

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Abstract. Kinetin, 6-furfurylaminopuirne, has been found to promote cell division in plants, and has also been reported to increase the rate of division of animal cells. In previous studies of its effect on the rate of division of *Paramecium caudatum*, some workers have found a stimulatory effect, whereas others have found inhibition. The effect of low concentrations of kinetin (K) on the rate of division was tested for another ciliate, *Blepharisma undulans*. No significant stimulation of division was found. A significant inhibition of division was found after treatment with 0.5 ppm K and with 1.5 ppm K.

The great problems of growth and morphogenesis still occupy their important places among the unsolved puzzles of biology. Pieces of the picture have emerged at intervals, and we now know something of what cell division and cell enlargement contribute. We know that these two processes are regulated, in part at least, by hormones.

One of the most recently discovered of these hormonal substances, kinetin, has been found to be able to cause, under certain conditions, the division of a cell which would not otherwise divide. Since this is the first chemical substance for which such a definite role as a cell division factor could be claimed, it has been the subject of great interest and intensive investigation. Kinetin was originally isolated from autoclaved DNA, and was later characterized as a substituted purine, 6-furfuryl-aminopurine, and synthesized (Miller et al., 1955, 1956).

As investigation of its properties has progressed, kinetin has been found to be active in a wide range of biological activities in plants, most of which probably involve cell division, but some of them also involving cell enlargement. In certain cases, however, it was found to have inhibitory rather than stimulatory effects on cell division (e.g., McManus, 1959, 1960; Brian and Hemming, 1957).

It has been of interest also to investigate its possible effects on animal cells. Fewer tests of this have been made, but here also, results are conflicting (Swann, 1958). The mechanism of action of any substance which can cause a cell to divide is of

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obvious importance to our understanding of both normal growth and growth of abnormal types such as cancer.

In an effort to obtain data on the effect of kinetin on animal cells, *Paramecium caudatum* were treated with kinetin in low concentrations by Guttman and Back in 1958 and 1960, and were reported to show an increased rate of cell division. But in similar experiments with *P. caudatum* in 1960 and 1961, McManus and Sullivan found an inhibition of division after treatment in the same concentrations and under similar conditions.

The experiments reported here were undertaken in order to get additional data on the action of kinetin on animal cells by treatment of an organism different from but similar to the previous test animal, *Paramecium*. *Blepharisma undulans* resembles *Paramecium* rather closely in its general morphology and physiology, and in the degree of complexity of its organelles and pellicle. It was used here as the test animal.

Materials and Methods

The strain of *Blepharisma undulans* used was taken from a contaminant in a myxomycete culture which had developed on wood with an overgrowth of moss. A clone was derived from one individual, and the clonal stock was maintained from this.

Preliminary experiments were done to determine the optimum medium for culturing the protozoa. They thrive best on solid food, but the particles of nutrient were hard to distinguish from the animals in counting, so a satisfactory fluid medium was sought. Of those tried, growth was best in an extract of tropical fish food. This was prepared by allowing one gram of the fish food granules to stand at room temperature for 24 hours in a beaker containing 250 ml distilled water. The supernatant was then decanted and sterilized and used as the nutrient.

The stock clone was kept in the dark in an incubator at 28°C $\pm$ 2°C in the fish food medium. For each experiment, an individual from the clone was isolated in a depression slide in the fish food medium. After two divisions, the four resulting individuals were placed separately into four depressions on slides. To one was added the control meduim, and to each of the others kinetin was added in a different concentration. One control thus served for comparison with an animal treated with each of three kinetin (K) concentrations, all being immediately derived from the same parent animal, and all being kept under identical conditions except for the concentration of K.

The depression slides were kept in a moist chamber in the incubator, and after 24 hours the number of animals present in each depression was counted and recorded. At least two animals

from each depression were transferred singly to new depressions, so that at the beginning of the second 24 hours there was again only one *Blepharisma* per depression. If no division had occurred, the animal was transferred to fresh control or treatment solution of the same concentration as it had been in. This procedure was repeated every 24 hours for three days. The experiment was then discontinued, and another begun with a new individual from the stock clone.

The kinetin was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and was kept under refrigeration both in the dry condition and after the solutions were made up.

The test slides were prepared by placing the control animals in depressions with two drops of distilled water and two drops of fish food extract. In the depression for the 0.5 ppm K treatment were placed two drops of the fish food extract and two drops of a 1 ppm aqueous K solution. For the 1 ppm treatment, equal parts of fish food extract and a 2 ppm K solution were used, and a 3 ppm K solution was similarly used for the 1.5 ppm K treatment. Droppers of the same bore were used for all solutions in making these dilutions.

Results

The results of a series of experiments carried out in November of 1961 are given in Table 1, which shows the mean number of divisions per 24-hour period of *Blepharisma undulans* treated with K in concentrations of 0.5 ppm, 1.0 ppm, and 1.5 ppm as compared with untreated controls.

Table 1. Mean Numbers of Divisions Per 24-Hour Period in *Blepharisma undulans* Treated With Kinetin As Compared With Untreated Controls.

Number of 24-hr. comparisons	Control	s	Concentration of K in pppm 0.5	1.0	1.5	t value	Level of significance
70	2.3	0.773	1.75			5.7	0.001
85	2.05	0.891		2.00		9.096	1.0
78	2.14	0.925			1.87	2.5	0.01

Seventy comparisons were made between organisms treated with 0.5 ppm K and controls immediately derived from the same parent individual. The mean number of divisions in the controls was 2.3 per 24-hour period, with a standard deviation of 0.773. The mean number of divisions per 24-hour period in animals treated with 0.5 ppm K was 1.75. Student's t-test for significance of the difference gives a t-value of 5.7, which is highly significant (at the 0.001 level).

Similarly compared, 85 animals treated with 1.0 ppm K showed no significant difference in the mean number of divisions per 24-hour period from untreated controls.

Seventy-eight comparisons between animals treated with 1.5 ppm K and controls showed a mean number of 2.14 divisions

for controls and 1.87 for those treated. The t-value of 2.5 indicates that the difference is significant (0.01 level) and K at this concentration was inhibitory to cell division.

A second series of experiments was conducted in December of 1961 and January of 1962. Table 2 shows the results of this series.

Table 2. Mean Numbers of Divisions per 24-Hour Period in *Blepharisma undulans* Treated With Kinetin as Compared With Untreated Controls.

Number of 24-hr. comparisons	Control	s	Concentration of K in ppm 0.5	1.0	1.5	t value	Level of significance
75	1.985	0.824	1.903			0.05	1.0
66	1.809	0.244		1.901		−0.31	0.85
82	1.986	0.821			2.086	−0.11	0.9

In this second series, the mean numbers of divisions in the control animals is somewhat lower than those of the first series, which may represent different stages of the annual cycle of activity in division rate reported for this species by Richards and Dawson (1927). The mean number of divisions for animals treated with 1.0 and 1.5 ppm is slightly higher than the means for their controls, but the t-test shows that the difference is not significant.

Only in the case of the first series of treatments with 0.5 ppm K and with 1.5 ppm K was the rate of cell division significantly changed. The change here was in the direction of inhibition rather than stimulation of cell division.

Discussion

Blepharisma undulans is a ciliate of the order Spirotricha and the family Spirostomidae (Kudo, 1960). It is elongate, often pyriform, with a narrowed anterior peristome and an undulating membrane along the right edge in front of the cytostome. Its pellicle is elastic. It is densely ciliated, but without cirri, and the cilia are arranged in a pattern of longitudinal rows. The cytopharynx is directed posteriorly and twisted to the right, and there is a contractile vacuole posteriorly in the body. The cytopyge is a rectangular region of undifferentiated ectoplasm free of cilia and granulation along the left side of the anal pole. All food and fluid vacuoles empty here (Moore, 1934).

The animal is usually pink or lavender due to the presence in its outer layers (whether ectoplasm or pellicle is disputed) of granules containing zoopurpurin. Strong light bleaches the pigment so that the bright rose color is maintatined only if the animals are kept in the dark. The findings of Giese (1938) on the development and maintenance of the color of the animals were confirmed here.

The size of *Blepharisma undulans* is ordinarly given as about 150μ in length by 60μ in width, but there can be great variation

from this size. Cannibalism has often been noted in this species, and can be induced by changing the food. If only *Tetrahymena* or *Colpidium* are available, the mouth parts of *Blepharisma* enlarge and they may ingest so many of the small animals that they become giants. They are then able to ingest other *Blepharisma*, and may become grossly misshapen. The animals may become twisted and their mouth parts may be strikingly enlarged and rotated by 180°. The other organelles also enlarge. The undulating membrane becomes very prominent and may protrude 25μ from the mouth, elongating to as much at 100μ so that it is able to enfold small animals on which it preys. The macronucleus also enlarges correspondingly with this excessive feeding.

When cannibalistic giants reproduce, they may give rise to other giants, or as many as 11 small blepharismas may be produced in quick succession. Chains of animals are sometimes seen. Chains of macronuclei have also been reported, and some authorities seem to relate this to the cannibalistic behavior (e.g., Manwell, 1961). However, in certain strains at least, there are vegetative nuclei consisting of a chain of a dozen or more nodes similar to the macronucleus of *Stentor coeruleus*. Just before the division cycle, these nodes fuse into three masses, of which the central mass disappears and two terminal ones fuse. This final fusion mass is the macronucleus, which elongates and divides to form the daughter macronuclei (Weisz, 1949).

Cannibalism has often been noted in the present study of *Blepharisma undulans*, and in our original cultures from which the clones were taken we often found misshapen giants. During the experiments cannibalism probably did not occur in the depression slides, however, because ample food was available and the marked difference in size and shape of the organisms would have been apparent. The slides were examined at least once a day, and no such difference was noted. Conjugation was also observed in the original cultures and sometimes in the stock clones, but was not observed in the depression slides during the experiments.

A yearly cyclic rhythm has been noted in the rate of division of *Blepharisma undulans*, of which the maximum is reported for July (Richards and Dawson, 1927). In our experiments of November and December, 1961, the mean numbers of divisions in control animals are slightly higher than those obtained in the tests of January, 1962. The cultures were kept in the dark, at a constant temperature, and were removed from darkness only for manipulation and counting. It was noted that the rate of division of both control and test animals did sometimes tend to fluctuate together, so that division rates for both seemed higher at the

beginning of the month, for instance, than at the end. No definite cycle of such short duration could be identified, however.

In the two series of experiments reported here we have again been unable to find evidence of a stimulatory effect by kinetin on division of animal cells, under conditions described. This correlates well with the previous findings of McManus and Sullivan (1960, 1961) that kinetin in low concentrations has no stimulatory effect on the rate of division of *Paramecium caudatum*, and again fails to confirm the stimulatory effect reported by Guttman and Back (1958, 1960).

The present studies have shown, in addition, that because of its endogenous rhythm of activity in cell division and its cannibalistic behavior, *Blepharisma undulans* is less satisfactory than *Paramecium caudatum* as a subject for studies of this kind. It may also be less sensitive to the action of exogenous growth regulators.

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