from the stimulus control voltage itself and amplified, and its phase could be controlled without change of amplitude by a circuit such as may be found in *Waveforms* (*3*).

One change in Brown and Forsyth's sequence may yet reveal an intracycle effect, namely, the interchange of light and dark phases. The "Metacontrast" (*4*) effect may enhance flicker enough to depress the curves noticeably in the region of small values of A. For best discrimination of change of threshold with interchange of light and dark phases, the presentation could be switched back and forth between the two modes for each setting of A. The difference would certainly be slight, but might be noticeable, especially at lower luminances and frequencies. If noticeable, the effect would qualify the hypothesis of independence of threshold of Fourier components.

Finally, it is possible that an observer could satisfy himself rather directly that he is responding to one or the other of the two Fourier components, for values of A and B near the intersection of the two curves. If B were set slightly greater than P, there should be two nearly equal values of A for which flicker is observed. The flicker frequencies should differ by a factor of 2, very nearly—a difference large enough to be discriminable.

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References

1. C. R. Brown and D. M. Forsyth, *Science* **129**, 390 (1959).
2. H. de Lange, *J. Opt. Soc. Am.* **48**, 777 (1958).
3. B. Chance *et al.*, *Waveforms*, vol. 19 of Mass. Inst. Technol. Radiation Lab. Ser. (McGraw-Hill, New York, 1949), p. 136.
4. M. Alpern, *J. Opt. Soc. Am.* **43**, 648 (1953).

4 May 1959

Competitive Antagonism between Kinetin and 8-Azaguanine in Polytoma uvella

Abstract. The enhancement of mitotic activity by kinetin is proportional to the dosage from 0.003 to 0.03 μg/ml. This effect is competitively antagonized by 8-azaguanine. The suppression can be reversed by a fresh supply of kinetin.

Kinetin (6-furfurylaminopurine) has been described as a substance which induces and stimulates mitotic divisions in tobacco pith tissue cultures (*1*). Furthermore, it could be shown that, in such kinetin-treated cells, doubling of deoxyribonucleic acid occurs prior to mitosis (*2*). In the course of studies on virulence changes in *Pneumococcus* and *Brucella* it was found that either deoxyribonucleic acid breakdown products or kinetin caused a selective multiplication of virulent cells within a mixed population (*3*). Stimulation by kinetin has been demonstrated in several other biological systems, such as Yoshida sarcoma cells (*4*), *Paramecium caudatum* (*5*), *Euglena gracilis, Ochromonas malhamensis,* yeasts (*6*), *Polytoma uvella* (*7*), and various plant tissues (*8*).

Since 8-azaguanine is known to inhibit mitotic divisions (*9*), this purine derivative was tested for an antagonistic effect on the mitosis-stimulating activity of kinetin (*10*). *Polytoma uvella* (WH strain) was chosen as a test organism. This phytoflagellate is a representative of the chlorophyll-free Volvocales; details of its culture and life cycle have been described elsewhere (*11, 12*). The test organism becomes multinucleate prior to cell division; experimental data were therefore collected as nuclear counts. Enhancement of mitosis is expressed as percentage increase of nuclei in treated cultures over untreated cultures after 18 to 22 hours, when the kinetin effect is most pronounced; that is, a 20-hour culture contained 610,000 nuclei per milliliter, while the corresponding kinetin-supplemented culture (0.06 μg/ml) had an increased nuclear count of 1.340 million per milliliter. This is expressed as 120 percent enhancement. Within the range from 0.003 to 0.03 μg/ml, the kinetin effect is proportional to the dosage (*11*). In all the experiments described, 8-azaguanine was used at a level of 10 μg/ml or less; at these levels it is noninhibitory with respect to *Polytoma.*

In the experiments performed to demonstrate antagonism, 8-azaguanine and kinetin were tested simultaneously in varying dosages (Table 1). It may be seen that the degree of suppression of kinetin enhancement depends on both the level of kinetin and the level of azaguanine. These findings indicate a competitive antagonism.

We have also been able to demonstrate the reversibility of the antagonistic effect. A set of cultures grown for 18 hours with 0.3 μg of azaguanine and 0.03 μg of kinetin per milliliter exhibits a suppression of 48 percent. Upon the addition of a fresh kinetin supplement (0.1 μg/ml), the suppression is markedly relieved by the 20th hour and is no longer evident at the 22nd hour.

Table 1. Suppression of kinetin effect by various dosages of 8-azaguanine.

Item	Percentage at various concentrations of kinetin					Av. of suppression (%)
	0.003 μg/ml	0.006 μg/ml	0.01 μg/ml	0.02 μg/ml	0.03 μg/ml	
Effect	+45	+65	+80	+96	+115	
			*Azaguanine + kinetin (1:1)**			
Reduced effect	+32	+50	+59	+75	+93	
Suppression	29	23	26	23	19	24
			Azaguanine + kinetin (10:1)			
Reduced effect	+20	+34	+39	+45	+60	
Suppression	56	48	51	53	48	51
			Azaguanine + kinetin (100:1)			
Reduced effect	+ 8†	+15	+18	+25	+32	
Suppression	(82)	77	78	74	72	75

* The corresponding molecular ratios are 1.5:1; 15:1; 150:1.
† Enhancement of 10 percent or less is of no statistical significance.

The fate of 8-azaguanine in living systems has been the topic of numerous studies, recently reviewed by Markham (*13*). This antimetabolite may replace guanine in nucleosides, in nucleotides, and in nucleic acids, or it may block a guanine-containing coenzyme system. Bergmann and Kwietny (*14*) have recently shown that kinetin is converted into 2,8-dihydroxy-kinetin by xanthine oxidase, and they discuss the possibility that "kinetin may react similarly to adenine in other enzyme systems." However, Kalckar *et al.* (*15*) found that inhibitors of xanthine oxidases did not influence the kinetin effect. We believe that kinetin is converted enzymatically to a still unidentified product, as is indicated by the observed competitive antagonism between kinetin and 8-azaguanine.

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References and Notes

1. C. O. Miller, F. Skoog, F. S. Okumara, H. von Saltza, F. M. Strong, *J. Am. Chem. Soc.* **78**, 1357 (1956); N. K. Das, K. Patau, F. Skoog, *Physiol. Plantarum* **9**, 640 (1956).
2. K. Patau, N. K. Das, F. Skoog, *ibid.* **10**, 949 (1957).
3. W. Braun, *Ann. N.Y. Acad. Sci.* **66**, 348 (1956); *J. Cellular Comp. Physiol.* **52**, 337 (1958).
4. Y. Ogawa, Y. Abe, K. Fujioka, *Nature* **180**, 986 (1957).
5. R. Guttman and A. Back, *ibid.* **181**, 852 (1958).
6. J. Supniewski, J. Krupinska, H. Supniewska, B. Waclaw, *Bull. acad. polon. sci. Classe* VI **5**, 19 (1957); J. Supniewski, and T. Marczynski, *ibid.* **5**, 437 (1957).
7. F. Moewus, *Abstr. Intern. Congr. Microbiol. 7th Congr. Stockholm* (1958), p. 38.
8. A. Dmochowski, W. Maciejewska-Potapczykowa, E. Sempinska, *Acta. Soc. Botan. Polon.* **26**, 361 (1957); M. J. Olszewska, W. Maciejewska-Potapczykowa, E. Sempinska, *ibid.* **26**, 583 (1957).
9. A. Gellhorn and E. Hirschberg, *Cancer Research* **15**, suppl. 3 (1955); G. G. Giminiz and C. Bonmati, *Publ. Univ. Murcia* (1956).

10. This investigation was supported by a research grant (C-3786) from the National Cancer Institute, Public Health Service. Kinetin was kindly supplied by F. Skoog, of the University of Wisconsin; azaguanine was supplied by Lederle Laboratories.
11. F. Moewus, *Trans. Am. Microscop. Soc.* **78**, in press.
12. ———, *J. Protozool,* **2**, suppl., 7 (1955), F. Moewus and L. Moewus, *Trans. Am. Microscop. Soc.* **78**, (1959).
13. R. Markham, "Lethal synthesis," in *The Strategy of Chemotherapy* (Cambridge Univ. Press, New York, 1958), pp. 163-177.
14. F. Bergmann, H. Kwietny, *Biochim. et Biophys. Acta* **28**, 100 (1958).
15. H. M. Kalckar, N. O. Kjeldgaard, H. Klenow, *ibid,* **5**, 586 (1940); H. Klenow, *Biochem. J.* **50**, 404 (1952).
*Deceased.

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Generator Potential of Insect Chemoreceptor

Abstract. Depolarization of the receptor membrane was recorded in labellar chemosensory hairs of flies upon stimulation by sucrose or NaCl. On the other hand, hyperpolarization was recorded in the case of $CaCl_2$, quinine, and acetic acid, all of which had an inhibitory effect on the initiation of chemosensory impulses.

It is now generally believed that in all kinds of receptors sensory impulses are initiated by changes in graded local potential (generator potentials) which are evoked by adequate stimuli. As for taste receptors, generator potential has not been recorded in either vertebrates or invertebrates (*1*). Labellar chemosensory hairs of flies have a structure which makes it possible to record this primary physiological process of chemoreception.

The recording arrangement is shown at the top of Fig. 1. This is an improvement over earlier methods (*2*). The hair wall near the tip of a labellar chemosensory hair of *Calliphora vomitoria* was pierced with a microneedle. At this point the fluid surrounding the sensory fibers inside the hair made contact with the 0.125*M* solution of NaCl contained in a capillary, whose diameter was about 30 μ. Through a platinum wire inserted into this capillary, the electrical activities of the sensory fibers near the hair tip were fed into a direct-coupled amplifier. After these procedures, a stimulating solution contained in another capillary was brought into contact with the hair tip. Movements of this capillary were controlled by an electromagnet and synchronized with sweeps of an oscilloscope.

Figure 1*A* is the record obtained by stimulating a chemosensory hair with a 0.25*M* solution of sucrose. It may be observed that after a brief upward surge, a negativity (downward deflection) is sustained during the stimulation, accompanying a train of impulses. We conclude that this negativity, as well as the impulse, represents a physiological event but that the initial surge is an artifact, since the negativity and the impulse almost disappeared but the initial surge was still recorded after the hair was crushed at the recording point (Fig. 1*B*). In no hairs was a *train* of impulses without a sustained negativity ever recorded; the negativity increased in magnitude with increase in the strength of the stimulus. Thus, it can be assumed that this negativity is the generator potential (depolarization of the receptor membrane). Such a potential was also obtained with NaCl.

It is interesting to note, on the other hand, that a positivity (hyperpolarization of the receptor membrane) was

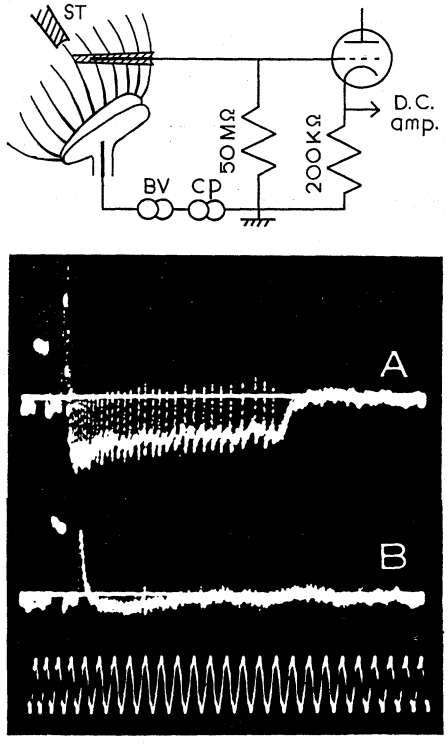

Fig. 1 (Top). Recording and stimulating arrangement. *ST*, Capillary containing a stimulating solution; *BV*, voltage source for balancing the potential difference between electrodes; *CP*, calibration pulse generator. (Bottom) *A*, Response to 0.25*M* sucrose; *B*, application of 0.25*M* sucrose after the same hair was crushed. Just before chemical stimulation, a rectangular calibration pulse of 1 mv (positive with reference to the ground) was applied. Time base, 60 cy/sec.

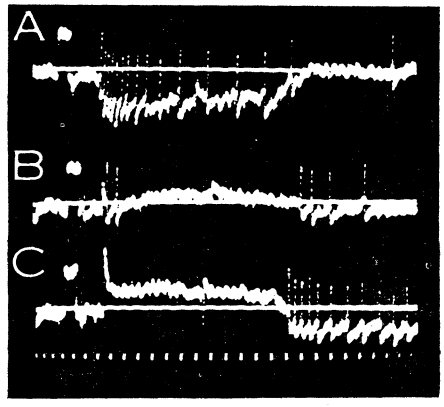

Fig. 2. Response of a hair to 0.25*M* sucrose (*A*), 0.25*M* sucrose + 0.05*M* $CaCl_2$ (*B*), and 0.05*M* $CaCl_2$ (*C*). Time, 1/60 sec.

produced by application of $CaCl_2$, acetic acid, quinine, and other compounds. These chemicals inhibited the initiation of impulses which might be evoked by sucrose or NaCl. One of these results is shown in Fig. 2. In Fig. 2*A*, a chemosensory hair responded to 0.25*M* sucrose in the same way as in Fig. 1*A*. When a mixed solution of 0.05*M* $CaCl_2$ and 0.25*M* sucrose was applied, a slight negativity with a few impulses was induced during the initial period of stimulation, but the negativity soon changed to a positivity, and the impulses disappeared. After the test solution was withdrawn, the positivity turned into a slight negativity, and impulses recurred (Fig. 2*B*). When a plain 0.05*M* $CaCl_2$ solution was used, the positivity and the afternegativity accompanied by the discharge of impulses were clearly observed (Fig. 2*C*). Enough other results have been obtained to show that the membrane potential of the chemoreceptor surface at the hair tip controls the initiation of impulses somewhere near the hair base (*3*).

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References and Notes

1. The existence of such generator potential has been postulated by many authors; for example, V. G. Dethier, "Chemoreceptor mechanisms," in "Molecular Structure and Functional Activity of Nerve Cells," *Am. Inst. Biol. Sci. Publ. No. 1* (1956).
2. E. S. Hodgson, J. Y. Lettvin, K. D. Roeder, *Science* **122**, 417 (1955); H. Morita, S. Doira, K. Takeda, M. Kuwabara, *Mem. Fac. Sci. Kyushu Univ. Ser. E* **2**, 119 (1957).
3. These studies were aided in part by the Scientific Research Fund of the Ministry of Education of Japan.

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