

much research is still needed to elucidate physiological roles of the various auxin components in the control of growth processes.

Introduction of the term "citrus auxin" as a new growth regulator is misleading since it implies that citrus tissues contain auxins distinct from the indolic auxins found usually in higher plant tissues. In view of the above-discussed evidence we feel that the use of the concept "citrus auxin" is not justified anymore and should be avoided in the future. "Auxins" in the broader biological sense, or the chemical names in case of chemically identified compounds, seem to be the adequate expressions to be used in studies of citrus as well as in studies of any other higher plant species.

Note added in proof: According to Lewis (10) the fluorescent material in young citrus fruits, maximum excitation wavelength of 350 nm and maximum fluorescence of 460 nm [citrus auxin, see table 1 in (1)], was found later to be scopoletin. Since scopoletin is not active in the *Avena* curvature test, it is not the auxin material that was found to be active in this bioassay (1). The nature of this material is not yet known (10).

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Cyclic Adenosine Monophosphate and Norepinephrine: Effect on Purkinje Cells in Rat Cerebellar Cortex

In many tissues, norepinephrine (NE) has been shown to accelerate the intracellular synthesis of adenosine 3,5'-monophosphate (cyclic AMP) (1). Hence it has been suggested that cyclic AMP might mediate the effects of NE.

It has been proposed that this mechanism could play a role in controlling the activity of rat cerebellar Purkinje cells (2); however it has been reported that NE exerts a weak and irregular depressant effect on cat Purkinje neurons (3).

In view of these reports, we tested these compounds with the usual microiontophoretic techniques (4) on 12 adult albino rats (Sprague-Dawley) either decerebrated or anesthetized with chloral hydrate (350 mg/kg, intraperitoneally); some Nembutal (25 mg/kg, intraperitoneally) was injected when required. Spontaneous activity and glutamate-induced firing of Purkinje neurons were in most cases (88 percent) depressed by NE (0.2M, pH 3 to 4) released iontophoretically with currents of 3 to 120 na for periods of 15 to 115 seconds. On the contrary, cyclic AMP (pH 7, or 6 in some experiments; 0.2M) applied with cathodal currents from 20 up to 225 na for 10 to 180 seconds, did not exhibit clear effects. Of 75 cells tested, 26 were excited, 43 showed no effect, and only 6 were depressed. The cyclic AMP effects were probably due to current artifacts, because iontophoretic injections of chloride (Cl⁻) resulted in a similar depression. In order to avoid gross current changes at the tip of the pipette, we applied a Cl⁻ current for a certain period of time after a cell had been found; when cyclic AMP was applied, the Cl⁻ current was switched off. Even then long application of cyclic AMP (up to 180 seconds) did not clearly depress cell firing.

Since it is known that methylxanthines inactivate (1, 5) the intracellular phosphodiesterase catabolizing cyclic AMP, the effectiveness of both NE and cyclic AMP was tested on 33 cells after intraperitoneal (2 rats, 10 cells) or intravenous (3 rats, 23 cells) injections of theophylline (dihydroxypropyltheophylline, 60 to 180 mg/kg). No significant change was observed.

Some of the above results are not in agreement with those of Siggins *et al.*

(2). A different electrical method of drug ejection (5, 6) cannot entirely account for the dissimilarity between their results and ours, since it would be unlikely that negative current effects would always balance out or overcome a supposed depressant effect of cyclic AMP, over the whole range of time and intensity of drug applications.

On the other hand, the negative observations after parenteral injections of theophylline support the proposition that, in certain circumstances, the pathway of degradation of cyclic AMP is insensitive to methylxanthines (7); other possibilities are that a theophylline-sensitive phosphodiesterase is not active, that it does not have access to the cyclic AMP generated in brain tissue, or that theophylline does not penetrate critical sites (8, 9). Finally the "classical" hypothesis of an indirect action of theophylline via the sympathetic system is not supported by a recent observation (10).

Although the data presented are at variance with some previous results (2) they are not surprising since the large cyclic AMP molecule is unlikely to be able to cross the cell membrane rapidly [as shown for the membranes of cardiac and skeletal muscle (8, 11)] and therefore could not easily reach its presumed intracellular site of action.

These observations do not permit any conclusion about the role of the cyclic AMP in the mediation of the depressant action of NE on Purkinje cells.

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As with other "negative" (1) results in this field, the inability of Godfraind and Pumain to differentiate effects of adenosine 3',5'-monophosphate (cyclic AMP) from "current artifacts" and to potentiate the inhibitory effects of norepinephrine (NE) on cerebellar neurons with a derivative of theophylline does not invalidate either our data or our interpretation. Analysis of their methods suggests that technical factors may account for the lack of success. Their techniques differ from our published experimental protocols (2-4) with respect to anesthesia, iontophoretic current controls, identification of Purkinje cells and assurance of their physiological rates of discharge, as well as the drugs used.

In particular, the use of large supplemental doses of pentobarbital (25 mg/kg) reduces Purkinje cell excitability (5) and has apparently required that Godfraind and Pumain use continuous applications of glutamate to evoke sufficient firing to test potentially inhibitory substances on many cells (6, discussion). This combination of drugs, not used in our studies, may lead directly to other complications: (i) the excitatory effects of glutamate may override any action of cyclic AMP, (ii) glutamate may have activated quiescent interneurons which we also find to be insensitive to cyclic AMP although slowed by NE, and (iii) glutamate excitation may make identification of Purkinje cells difficult since a high rate of induced activity could be confused with spontaneous, climbing fiber-evoked responses (7). Our reports (2, 3, 8) of cyclic AMP inhibition of Purkinje cells were based on responses observed in rats lightly anesthetized with halothane or chloral hydrate, in which spontane-

ously active Purkinje cells were positively identified by electrophysiological criteria.

Second, the implication is erroneous that application of Cl^- currents alone followed by application of cyclic AMP currents would eliminate the complications derived from gross polarizations of the iontophoretic electrode and that this Cl^- current would be a suitable control for polarizations occurring during application of cyclic AMP. The drug currents can be reliably controlled only by use of a continuous balancing current equal in magnitude and opposite in polarity to the sum of the ejecting and retaining currents flowing through the other barrels of the multibarrel pipette electrode (9). Such controls are of crucial importance because of the large iontophoretic currents frequently needed to observe effects with cyclic AMP. In our experience, passage of Cl^- current usually activates healthy Purkinje cells and can reverse the inhibitory effects of cyclic AMP [figure 2A in (2)].

Third, the failure to observe potentiation of cyclic AMP or NE responses after administration of dihydroxypropyltheophylline can scarcely invalidate our observations. This theophylline derivative gained clinical prominence chiefly because it has little or no effect on the central nervous system at doses as high as 400 mg/kg (10), presumably because of a relative inability to enter the brain. We have observed potentiation of the depressant effects of cyclic AMP and NE on Purkinje cells with the parent compound theophylline and with aminophylline, injected parenterally in doses that inhibit brain phosphodiesterase activity. Furthermore, we do not understand how substantive control observations could have been made by Godfraind and Pumain before each injection of the theophylline derivative, since they report studying a total of 33 cells in five animals, using multiple doses in each animal. We find that an effective dose of parenteral methylxanthine produces such long-lasting effects on both central and autonomic nervous systems that long-term testing of each Purkinje cell after a single dose of methylxanthine to an individual animal is required.

Moreover, our proposal that cyclic AMP mediates the inhibitory action of NE on Purkinje cells does not depend solely on the reported potentiations by parenteral methylxanthines, but also on

much published data not cited by our critics. We have also applied aminophylline and theophylline, as well as the papaverine class of phosphodiesterase inhibitors (11), by iontophoresis. Again, augmented depressant actions of cyclic AMP and NE on Purkinje cells were seen, even when the initial direct responses to cyclic AMP were ineffective or even slightly excitatory (3, 4). After iontophoresis of any of these phosphodiesterase inhibitors, the only effects produced by iontophoresis of cyclic AMP were depressions of spontaneous Purkinje discharge (3, 4). Furthermore, nicotinate and prostaglandins E_1 and E_2 (known to affect cyclic AMP levels in peripheral end organs) specifically and selectively block the action of NE on Purkinje cells (3, 4). It seems unlikely that this diverse array of pharmacological agents would consistently exert such a specific action against an alleged current effect of cyclic AMP.

We have reported that locally applied cyclic AMP, dibutyl cyclic AMP, and NE exert qualitatively identical and unique actions on both the membrane potential and resistance of Purkinje cells recorded with intracellular electrodes (12); each of these drugs causes hyperpolarization, frequently accompanied by increased input resistance. Depolarizations of the Purkinje cell membrane in response to cyclic AMP were never seen. Since cyclic AMP and NE are applied with currents of different polarity, it seems unlikely that the similar transmembrane responses can be attributed to current effects. Again, in studies on the neonatal Purkinje cell responses to iontophoresis of various substances before the onset of synapse formation (13), cyclic AMP and NE virtually always produced a depressant effect on the spontaneous activity. Such responses could frequently be observed by simply allowing cyclic AMP to diffuse from the iontophoretic barrel after removal of the retaining current.

Furthermore, Godfraind and Pumain cite the report by Kawamura and Proveni (14) that NE has only weak and irregular depressant effects on cat Purkinje cells. However, we find extremely potent depressant responses of these neurons of both NE and cyclic AMP (15).

The implication that extracellularly administered cyclic AMP cannot cross cell membranes is no longer tenable; exogenous cyclic AMP produces potent physiological effects in a variety of in-

tact cell types [see discussion (3)] and can be shown to cross cell membranes rapidly and routinely (16). Thus the highly supportive pieces of interlocking data now in hand suggest that the negative comments of our critics in no way weaken our hypothesis and therefore need not be considered further.

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References and Notes

1. By our calculation, Godfraind and Pumain find that nearly 60 percent of their tested cells do not respond to cyclic AMP, compared to 17 percent of our identified Purkinje cells. Negative results are common in microiontophoretic experiments [see discussions of H. McLennan, *Nature* 288, 674 (1970) and D. R. Curtis, A. W. Duggan, D. Felix, G. A. R. Johnston, *ibid.*, p. 676, as opposed to that of J. M. Godfraind, K. Krnjević, R. Pumain, *ibid.*, p. 675] and require cautious interpretation [see B. J. Hoffer, N. H. Neff, G. R. Siggins, *Neuropharmacology* 10, 175 (1971)].
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8. We have now tested 193 Purkinje cells with iontophoresis of cyclic AMP; 83 percent of these respond to cyclic AMP, 62 percent with depression of discharge, 14 percent with elevated firing rates, and 7 percent with mixed biphasic or reversible responses. It may be more than fortuitous that the percentage of our cells responding with depression equals the negative responses of Godfraind and Pumain.
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17. We thank Drs. Forrest Weight and Roger Nicoll for critical evaluation and Odessa Colvin for typing of the manuscript.

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Paramagnetic Ions in Zoisite

We have carried out a study of transition metal impurity ions in zoisite by electron paramagnetic resonance. Our results will be reported elsewhere (1), but, since they are considerably at variance with those of Ghose and Tsang (2), we present a preliminary account of them here. The measure-

ments were made with standard 3-cm and 8-mm spectrometers, each having full rotation facilities for the magnet and the specimen. Specimens studied included heated gem-quality and unheated natural crystals (3) from Tanzania.

All our evidence suggests the pres-

ence of Fe^{3+} , Cr^{3+} , Mn^{2+} , VO^{2+} , and V^{4+} ions. The various spin Hamiltonian parameters and the orientations of the magnetic axes are given in Table 1.

The single anisotropic line at a wavelength of 3 cm detected by Ghose and Tsang and attributed by them to Fe^{3+} is in fact due to Cr^{3+} . Its anisotropy from an effective spectroscopic splitting factor g of 2 to 4 labels it as a $-\frac{1}{2}$ to $+\frac{1}{2}$ transition in the $|\pm \frac{1}{2}\rangle$ doublet of an S (electronic spin) $= 3/2$ spin system with large crystal-field splitting. The presence of Mn^{2+} forbidden transitions along the magnetic z - and y -axes indicates that the cubic field and the lower symmetry tensors do not have the same principal axes (4). This result might be expected if the Mn^{2+} substitutes into a very low-symmetry Ca^{2+} site.

The assignment of the vanadium lines to VO^{2+} and V^{4+} (d^1) follows from the temperature dependence of the spectra and the absence of fine structure lines that would characterize V^{2+} (d^3). The small anisotropy in g at a wavelength of 3 cm and the absence of fine structure at the Q band precludes the possibility that the spectra are due to V^{2+} with large zero field splittings. Because the axes of the vanadium sites correlate strongly with those of the Al nuclear quadrupole tensors (5), we would allocate the VO^{2+} to Al substitutional sites.

Our experience in the field of magnetic resonance in mineralogy has led us to the conclusion that full rotation facilities for crystal and magnet, and the use of at least two widely separated frequencies, are essential for such studies.

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Table 1. Ion spin Hamiltonian parameters and orientations in zoisite.

Spin Hamiltonian parameters*	Orientation
$g_z = 1.948 \pm 0.001$, $A_z = 163.4 \pm 0.2$ $g_y = 1.968 \pm 0.001$, $A_y = 53.3 \pm 0.2$ $g_x = 1.942 \pm 0.001$, $A_x = 49.2 \pm 0.2$	$(\text{VO}^{2+})_1$ The z -axis is 39° from the b -axis, 7° from the (100) plane; the x -axis is in the (100) plane; four sites.
$g_z = 1.938 \pm 0.001$, $A_z = 161.7 \pm 0.2$ $g_y = 1.928 \pm 0.001$, $A_y = 52.5 \pm 0.2$ $g_x = 1.944 \pm 0.001$, $A_x = 45.2 \pm 0.4$	$(\text{VO}^{2+})_2$ The z -axis is in the (010) plane, 11° from the a -axis; the y -axis is parallel to the b -axis; two sites.
$g = 2.000 \pm 0.001$ $D = 0.787 \pm 0.004$ $E = 0.015 \pm 0.001$	Fe^{3+} The z -axis is parallel to the a -axis; the y -axis is parallel to the b -axis; one site.
$g_{\parallel} = 1.9705 \pm 0.0005$ $g_{\perp} = 1.975 \pm 0.001$ $D = 0.636 \pm 0.0003$ $E = 0.0277 \pm 0.0003$	Cr^{3+} The z - and y -axes are in the (010) plane, 45° from the a and c axes; two sites.

* The parameters may be defined as follows: g is the spectroscopic splitting factor; A is the hyperfine interaction constant; and D and E (in reciprocal centimeters) are crystal-field parameters.

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