

## References and Notes

1. We use the term plexus, as defined by Bullock and Horridge (2), to indicate nerve fibers, with or without cell bodies, in the skin and other peripheral structures.
  2. T. H. Bullock and G. A. Horridge, *Structure and Function in the Nervous Systems of Invertebrates* (Freeman, San Francisco, 1965).
  3. I. Beritov and M. Gogava, cited in (2), p. 756 [*Tr. Tbilis. Gos. Univ.* 27a, 1 (1945)]; H. Jordan, *Z. Biol. Munich* 41, 196 (1901).
  4. A. Bethe, *Allgemeine Anatomie und Physiologie des Nervensystems* (Leipzig, 1903).
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  6. F. B. Hoffman, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* 132, 43 (1910); D. F. Wilson and R. A. Nystrom, *Comp. Biochem. Physiol.* 26, 663 (1968).
  7. Weak and medium strength tactile stimuli (jets of seawater) were produced by the low (3 g) and medium (6 g) settings of a Water Pik apparatus or by a poke with No. 2 red sable brush (1 g). Strong stimuli were produced by the maximum setting (25 g) on the Water Pik, by vigorous brushing, or by poking with a glass or wooden rod (12 g). The force of the jet of seawater was measured by directing it under water, at a 2.54-cm-diameter target, 1.9 cm away from the nozzle. The target was attached to a force transducer by a 5-cm arm. The same system was used to estimate the force of the brush, by pressing the brush against the target.
  8. The less committal term "pinnule response" is preferable to pinnule reflex. A response that remains after the central nervous system has been removed should not be considered a reflex until it can be shown that sensory and motor neuronal elements are involved. It is not known whether the pinnule response is mediated by peripheral ganglia; by a peripheral nerve plexus; or by direct responsiveness of, and conduction through, muscle fibers.
  9. I. Kupfermann and E. R. Kandel, *Science* 164, 847 (1969).
  10. B. Peretz, *ibid.* 166, 1167 (1969).
  11. I. Kupfermann, V. Castellucci, H. Pinsker, E. R. Kandel, *ibid.* 167, 1743 (1970).
  12. The value of 40 percent is based on 11 experimental runs each consisting of two trials in which the gill contraction elicited when L7 was hyperpolarized was compared to the contraction when L7 was not hyperpolarized. In the relatively intact preparation the size of the reflex response tends to decrease throughout the course of an experiment. Therefore, an alternate estimate of the L7 contribution to the reflex was obtained by comparing the reduced response with L7 hyperpolarized to the average amplitude of the two gill responses taken during the trials immediately before and after L7 was hyperpolarized. This procedure yielded values of 36, 38, 47, and 83 percent for the contribution of L7. A similar procedure on one LD-G cell yielded a value of 36 percent.
  13. These experiments utilized a relatively intact preparation with a slit made in the neck region to externalize the abdominal ganglion (11). The stage on which the ganglion was pinned was modified into a small chamber that could be filled with 1 or 2 ml of a given solution with little leakage into the large chamber (3 liters) that held the animal. The level of the seawater in the main chamber was maintained slightly above the ganglion chamber, so that leakage consisted primarily of flow into the ganglion chamber. Probably because of leakage, synaptic transmission through the ganglion was not invariably blocked completely; even with a solution of pure isotonic  $MgCl_2$ , postsynaptic potentials, although always reduced, were not always completely abolished. In two additional experiments utilizing extracellular recordings from the genital, pericardial, and branchial nerves, we found that raising the magnesium concentration to four times normal blocked all efferent spike activity evoked by a tactile stimulus to the siphon. Since this concentration of magnesium did not block spike activity, it appears likely that all central afferent pathways from tactile receptors make chemical synaptic connections in the abdominal ganglion.
  14. The gill was removed from the animal and remained attached to the abdominal ganglion by means of the branchial nerve, which enters the efferent vein of the gill at its anterior insertion. The efferent vein was opened to allow visualization of the muscle fibers whose contractions contribute a significant part of the total gill-withdrawal reflex. K. Robinson (personal communication) has some anatomical evidence that the region of the efferent vein from which intracellular recordings were obtained is rich in muscle cells and is devoid of any neuronal cell bodies. These experiments on L7 and parallel experiments on LD-G will be described in detail elsewhere (H. Pinsker, T. Carew, K. Robinson, I. Kupfermann, E. R. Kandel, in preparation).
  15. In some cases stimulation of the gill seemed to trigger interneuron II (as evidenced by a burst of inhibitory postsynaptic potentials in L7) and led to a triggered or late "spontaneous" contraction of the gill (9, 10).
- clearly distinguishable from either the pinnule response or the gill-withdrawal reflex. It is thus possible that the gill sends an excitatory input to interneuron II.
16. H. Pinsker, I. Kupfermann, V. Castellucci, E. R. Kandel, *Science* 167, 1740 (1970).
  17. W. J. Crozier and L. B. Arey, *J. Exp. Zool.* 29, 261 (1919).
  18. H. de Lacaze-Duthiers, *C. R. Acad. Sci. Paris* 105, 978 (1887).
  19. C. L. Prosser, *Physiol. Rev.* 26, 337 (1946).
  20. We thank K. Kuwasawa for assistance, K. Hiltner for help on the illustrations, T. Carew and W. A. Spencer for their comments, and W. Hening for calibrating the Water Piks. Supported by PHS grants NS 09361, MH 15980, NS 07621, career development award MH 12240 to I.K., career scientist award MH 18,558 to E.R.K., and a Canadian Medical Research Council fellowship 100-2C-88 to V.C.

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## Auxins in Citrus: A Reappraisal

Beginning with a report in 1963 (1), a group of scientists from the Citrus Research Center at Riverside, California, published a series of papers dealing with a new, natural growth-promoting substance that has been found in young citrus fruits. Results obtained through thin-layer and column chromatography, paper electrophoresis, and spectrofluorimetric determinations showed that the new compound could not be indoleacetic acid (IAA) and suggested that it was nonindolic. Being unable to find any indolic auxins in citrus fruits, Khalifah, Lewis, and Coggins called the new compound "citrus auxin" and considered it to be of physiological significance in citrus and probably also in additional plant species (2).

Accumulating physiological and chemical evidence seems to warrant a reappraisal. Bioassay determinations revealed the presence of considerable auxin activity in numerous citrus tissues (3). An IAA-oxidase system was detected in roots and aerial parts of citrus seedlings, showing specificity toward IAA (4). Vigorously growing Eureka lemon shoots contain a single auxin component which copartitions and cochromatographs with labeled IAA and migrates to the same  $R_F$  as synthetic IAA in eight solvent systems in paper chromatography (5). The difficulties in obtaining the chromogenic reactions typical to IAA were also overcome by employing solvent partition followed by a two-dimensional run on thin-layer chromatography (TLC), with up to 100 g of fresh material per TLC plate. Labeled IAA markers and biological auxin activity were recovered from the zones that responded to

chromogenic sprays on parallel plates (6).

Flower parts, including ovaries, showed relatively high auxin activity, as determined by bioassay (3). Extracts from fruits at later developmental stages evinced, on purification, several zones with auxin activity, but did not seem to contain detectable amounts of IAA (7). However, Khalifah (who himself belonged originally to the "citrus auxin" team) showed that citrus fruitlets incorporated [ $^{14}C$ ]tryptophan into IAA (8).

Direct and complete evidence for the existence of indolic auxins in citrus fruits has now been provided through extensive purification followed by chemical and physical identification. An extract from young fruitlets of *Citrus unshiu* (satsuma orange), a week after bloom, was purified by solvent partition followed by numerous steps of column, paper, and thin-layer chromatography and by countercurrent distribution, yielding several zones which respond to Ehrlich's reagent and show biological activity in the *Avena* curvature bioassay. The active components were identified by gas-liquid chromatography, and by ultraviolet, infrared, and mass spectra. The young fruitlets were shown to contain IAA [0.5 to 1.0 mg/kg (fresh weight)] and indoleacetamide [5.0 mg/kg (fresh weight)] (9). Only traces of IAA and indoleacetamide could be found in 2-month-old fruits, but neither fruitlets nor older fruits revealed the presence of biologically active auxins which had the properties ascribed to "citrus auxin" (9).

It seems, therefore, that the existence of indolic auxins in citrus tissues is now well documented, even though

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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11. The cooperation of Dr. Lewis is gratefully acknowledged.

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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<sup>-</sup>) resulted in a similar depression. In order to avoid gross current changes at the tip of the pipette, we applied a Cl<sup>-</sup> current for a certain period of time after a cell had been found; when cyclic AMP was applied, the Cl<sup>-</sup> 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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