nism do exist at the spinal level of the central nervous system. This corresponds with earlier findings[7] that a restoration of thermoregulatory mechanisms may take place, to some extent, in chronic spinal animals.

Zusammenfassung. Bei spinalen wie auch bei intakten, leicht narkotisierten Kaninchen konnte durch Kühlung des Rückenmarkes Muskelzittern ausgelöst werden. Im Vergleich zu den intakten Tieren war der Effekt der Rückenmark-Kühlung bei spinalen Tieren schwächer, und die Temperatur zu Beginn und bei Beendigung der elektromyographisch erfassten Aktivität war niedriger, bzw. streute in einem weiteren Temperaturbereich. Die während des Kältezitterns intakter Tiere häufig zu beobachtenden phasischen Aktivitätsschwankungen im Elektromyogramm waren bei spinalen Tieren nur selten nachweisbar.

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W. G. Kerckhoff-Institute of the Max Planck-Gesellschaft, Bad Nauheim (Germany), 8th December 1966.

[7] R. Thauer, Pflügers Arch. ges. Physiol. *236*, 102 (1935).

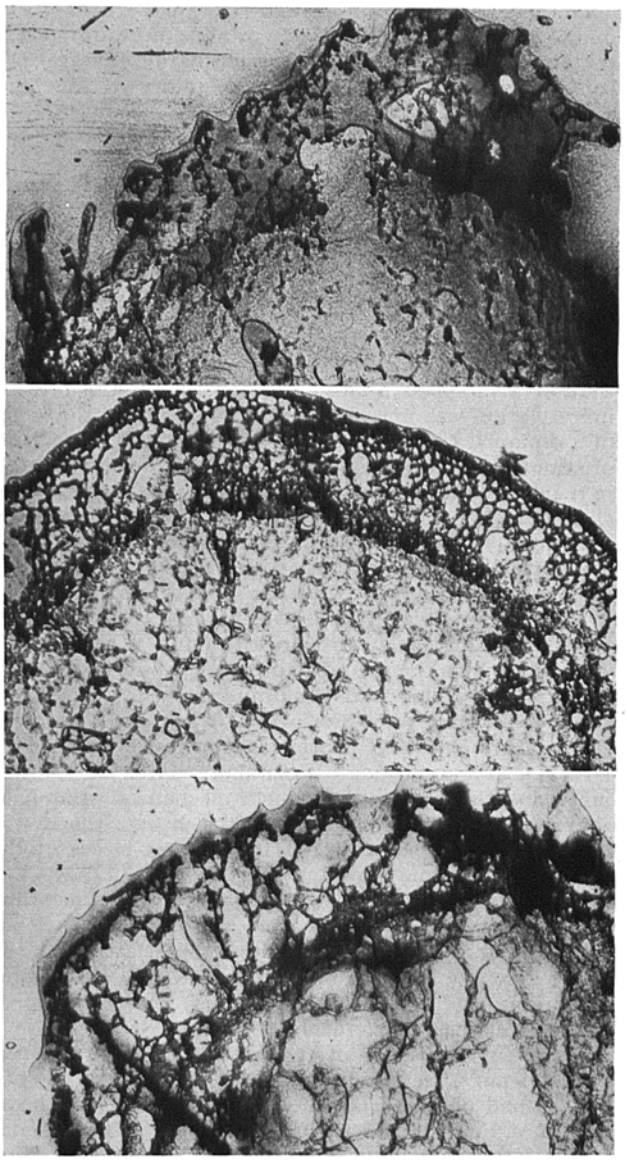
Autoradiographic Evidence of Tritiated Indolyl-3-Acetic Acid in Epicotyl Tissue of *Phaseolus coccineus*

One of the difficulties involved in obtaining autoradiographic evidence of the tissue in which indolyl-3-acetic acid (IAA) is translocated in a polar manner is the fact that the polar transport mechanism is saturated at very low concentrations of IAA[1]. Therefore, radioactively-labelled IAA with a high specific activity is required in order to obtain sections with radioactivity above background. Moreover, because IAA is soluble in water and alcohols, the usual procedures of fixing, embedding and autoradiography cannot be used.

Tritium-labelled IAA (generally labelled), with a specific activity of 112 mc/m mole was used in these experiments in an attempt to show the tissues involved in translocating this compound. Segments of epicotyl 6 mm in length were cut from 1 cm below the hook of 7-day-old dark-grown seedlings of *Phaseolus coccineus* (Scarlet Runner beans). All manipulations of seedlings were carried out using a safelight having peak transmittance at 525 nm and cut offs at 500 and 560 nm. Donor concentrations of $10^{-4}M$ IAA were applied in cylindrical agar blocks using a series of treatments. The treatments included normal and horizontal orientation of the tissue segments and different durations of diffusion.

In order to prevent leaching and to localize the radioactivity incorporated during the treatments, it was necessary to freeze the tissue and to section it without it coming in contact with any solvent, and without it being thawed. Before freezing, the tissue was marked with ink at positions for which sections were desired, ca. $^1/_4$, $^1/_2$ and $^3/_4$ of the way along the segments.

The segments were flash-frozen in isopentane cooled in dry ice. The frozen segments were transferred, packed in dry ice, to a cryostat where they were maintained overnight at $-20\,°C$. They were then sectioned with a microtome contained in the cryostat. Using a camel's hair brush, the sections (ca. 14μ thick) were transferred from the microtome knife to slides previously treated with Haupts solution. The slides were transferred to pre-cooled flasks connected to the freeze drying apparatus. The flasks containing the open slide boxes were immersed in an ice and salt bath below $-10\,°C$ to maintain the tissue



Tissue radioautographs of sections of *Phaseolus coccineus* epicotyl segments after treatment with IAA-H^3 from donor agar blocks (X 45). Top: tissue oriented normally, diffusion time 3 h. Middle: same as above, but the sections were fixed instead of being developed. Bottom: tissue laid horizontally, diffusion time 3 h.

[1] M. H. M. Goldsmith and K. V. Thimann, Pl. Physiol., *37*, 492 (1962).

frozen whilst being dried. When dry, the slides were stored in slide boxes containing Drierite, and the boxes were sealed with tape.

Thin films of Kodak NTB 3 emulsion were prepared by the method described by MILLER et al.[2]. The slides were coated with the film and were exposed for 3 months at room temperature in the sealed slide boxes. At the termination of the exposure time, some of the slides were fixed, instead of being developed, to serve as controls.

Macroscopic examination of the slides showed that the zones of greatest blackening of the emulsion occurred in regions corresponding to the epidermis and cambial regions (Figure). However, label was distributed generally throughout the sections. In sections taken from regions near the donor blocks, very dark spots corresponding to label in xylem patches were observed. Sections which had been administered label for 3 h were generally darker than those administered label for only 1 h. All the treatments were darker than the controls or background. Microscopic examination showed that the darkening was due to silver grains but did not provide any information not apparent from macroscopic examination. Taking into consideration the high degree of solubility of IAA and the probability that its polar transport occurs by a secretive mechanism the evidence of labelled IAA dispersed generally throughout the sections is not surprising.

Résumé. Des tronçons d'épicotyles de plantules de *Phaseolus coccineus* ont été traités à l'acide indole acétique (AIA) marqué au tritium. Ces tronçons ont été soumis au «freezing-drying», puis réduits en coupes minces, en évitant tout contact avec un solvant quelconque. L'étude autoradiographique révéla la présence de l'AIA dans tous les types de tissu vivant.

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Department of Plant Science, University of Alberta, Edmonton (Alberta, Canada), 24th October 1966.

[2] D. L. MILLER JR., G. E. STONE and D. M. PRESSCOTT, *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), vol. 1, p. 371.

Physiology of the Heart of *Cingalobolus bugnioni* (Diplopoda: Myriapoda)

In view of the fact that scarcely anything is known about the physiology of the hearts of myriapods, except that of a chilopod *Scolopendra morsitans*[1], the present investigation was undertaken on the nature of the heart of a diplopod *Cingalobolus bugnioni* Carl, and the effects of stimuli, pH and drugs on it. The hearts were isolated in a saline containing sodium chloride 0.63 g, potassium chloride 0.025 g, calcium chloride 0.025 g, and glucose 0.2 g in 100 ml of distilled water and the pH adjusted to 6.3 with phosphate buffer as the haemolymph of the diplopod was found to be on the acid side of neutrality in concordance with MALUF[2]. Heart-beat after isolation was slow and irregular for nearly 10 min. On recovery, it showed a high degree of automatic movement with incessant rhythmic and simultaneous beat throughout the myocardium, at a rate of 52–61 beats/min at room temperature (29°–31 °C) without rest pauses. After 10–12 h its rate and amplitude fell gradually. After temporarily inhibiting the heart-rate by such mechanical stimuli as shaking the saline or pressing the heart, there was acceleration of the rate of beat. Isolated pieces of the heart beat for some time.

Both increasing and decreasing the pH of the saline with phosphate buffers depressed the heart-rate, and increase of temperature accelerated the heart-rate. The upper limit was 84–93 beats/min at 43 °C; the lower limit on cooling was 7–10 beats/min at 5 °C, but it recovered on increasing the temperature.

Faradic stimulation of the brain and ventral nerve cord did not affect the rate of beat of the heart in situ. It also had no effect on direct application to the heart up to 2 V. Further increase in the intensity of the stimulus in the latter case stopped the heart-beat. On switching off the current, the heart-beat reappeared after 10–15 min. Electrically stimulated hearts were not used for drug experiments.

The effects of various drugs were observed on hearts both in situ and isolated. Acetylcholine at 10^{-5} dilution or stronger depressed the rate of beat, but the heart recovered after washing with saline. At higher dilution acetylcholine had no effect. By first treating hearts with higher dilutions and then gradually increasing the concentration, tolerance to acetylcholine up to 10^{-4} dilution was observed. The action of acetylcholine was potentiated by physostigmine (10^{-4}). Histamine (10^{-4}) accelerated the heart-rate and antagonized the action of acetylcholine. Adrenaline (10^{-5}) accelerated, atropine (10^{-4}) inhibited and ether (half and fully saturated) had no effect on the heart-beat. On many occasions the best agent for reviving the collapsing heart was warm saline, which was added in drops to the heart container. The time of recovery varied from 5–15 min.

According to the classification of hearts by NEEDHAM[3], the diplopod heart may be put among the myogenic types. It will be interesting to note in this context, that the chilopod heart is neurogenic[1]. A detailed account of this work will be published elsewhere[4].

Zusammenfassung. Herzphysiologische Untersuchungen am Diplopoden *Cingalobolus bugnioni* ergaben: Während Histamin und Adrenalin die Herzfrequenz beschleunigen, wirken Acetylcholin und Atropin hemmend. Es wird angenommen, dass das Herz dieser Diplopoden vom myogenen Typus ist.

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[1] G. SUNDARA RAJULU, J. Anim. Morph. Physiol. *13*, 34 (1966).
[2] N. S. R. MALUF, Q. Rev. Biol. *14*, 149 (1939).
[3] A. E. NEEDHAM, Nature *166*, 9 (1950).
[4] My thanks are due to our Principal Dr. A. CHIDAMBARANATHAN CHETTIAR for his interest in this investigation. The technical assistance rendered by Mrs. S. GOWRI is much appreciated.