Production of Embryos on the Stem of *Ranunculus sceleratus* L.

The floral buds of *Ranunculus sceleratus* excised at the primordia stage (showing sepal, petal and stamen primordia) proliferated into an extensive callus on a modified White's medium supplemented with coco-nut milk (10% v/v) and indole-3-acetic acid (1 ppm). Subsequently, the callus differentiated into embryo-like structures (designated embryoids) which developed into seedlings, both in situ and when excised and transferred to a fresh medium.

A remarkable feature of the 3- to 4-week-old seedlings is that they themselves show supernumerary embryos at the radicular end or along the surface of the stem (Figures 1, 2). The development of embryos is non-synchronous and several developmental stages may be seen on the same seedling. The mature embryos possess a radicle-plumule axis and two (sometimes three) cotyledons characteristic of the embryos which develop from normally fertilized ovules. The embryos germinate in situ and also when excised and transferred individually to a fresh medium.

Portions of the stem of the seedlings, free from embryos, were transferred to White's medium fortified with coconut milk (10% v/v). In 2-week-old subcultures, the explants were already studded with new embryos. Microtome sections revealed that the accessory embryos originate from the epidermal cells. Structurally and functionally these embryos are similar to zygotic embryos. To the best of our knowledge this is the first report of the origin of embryos from the epidermal cells of the stem which provides an additional proof of totipotency of the cells[1].

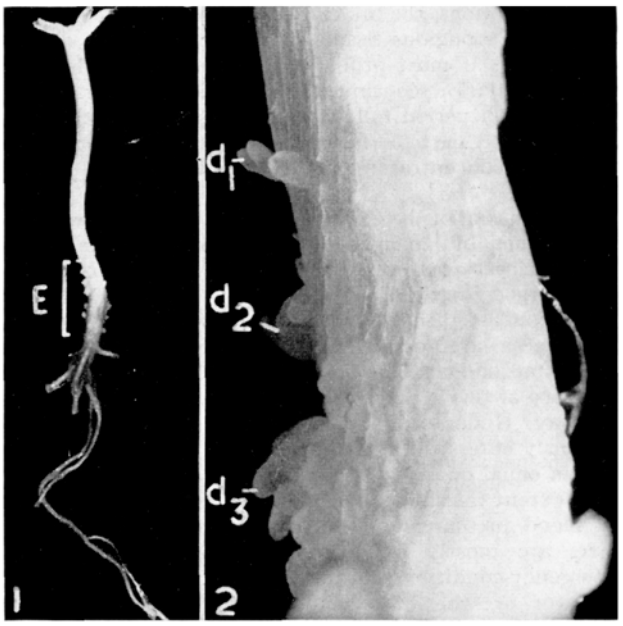

Fig. 1. Seedling with lower portion of the stem studded with accessory embryos. × 5.

Fig. 2. Enlarged view of the portion marked E in Figure 1; note especially the embryos at d_1, d_2, d_3. × 65.

Résumé. Les embryoïdes se développant à partir du bourgeon de calle de *Ranunculus sceleratus* deviennent des plantules. Ces plantules produisent des embryons surnuméraires à l'extrémité radiculaire ainsi que tout le long de la surface de la tige. Les études anatomiques révèlent que ces embryons sont d'origine épidermique. C'est une nouvelle preuve de la totipotence des cellules.

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Department of Botany, University of Delhi (India), March 13, 1965.

[1] Our thanks are due to Prof. P. Maheshwari for his advice and encouragement.

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On the Presence of an External Hemal Pore in *Lymnaea stagnalis* L.

When specimens of the pond snail, *Lymnaea stagnalis* L., are mechanically irritated, they extrude several drops of fluid. This can be demonstrated when a snail has been kept out of water on filter paper for some minutes, by pushing a glass rod against the exposed parts. It can then also be observed quite easily that the fluid escapes from the pneumostome.

The present study was undertaken to determine the nature and the place of exit of this fluid. When studied under the microscope it appeared to contain many amoebocytes, which shows that the main part of the fluid does not consist of urine and of water present in the lung cavity. Therefore it was concluded that the fluid, at least partly, must be blood.

This was confirmed by the fact that when snails were injected in the head or foot with 0.2–0.3 ml of methylene blue or neutral red solutions the fluid extruded via the pneumostome within 1 sec after the irritation by the injection had the colour of the dye administered.

At first it was thought that the loss of blood had to be explained either by rupture of the pericardium wall, so that blood enters the respiratory cavity through the renopericardial canal and the kidney tube, or by the bursting of the thin-walled blood vessels in the roof of the lung. During careful macroscopic and microscopic examinations, however, no arguments in favour of these two suppositions could be obtained.

The problem was solved in the following way. Full-grown snails (shell length over 30 mm) were injected with 0.2 ml of Indian ink. After the extrusion of black fluid, the animals were narcotized[1] and the lung cavity was

[1] Method described by J. Lever, J. C. Jager, and A. Westerveld, Malacologia *1*, 331 (1964).

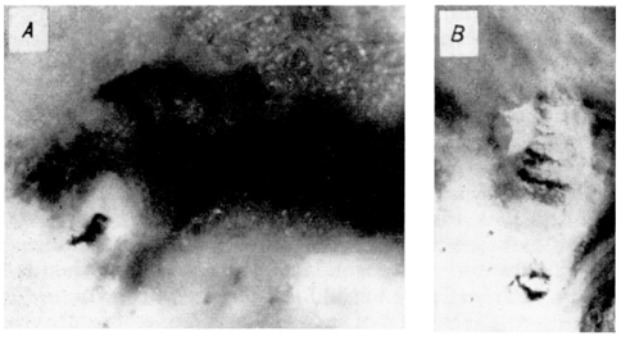

Fig. 1. Part of the lung wall near the renal pore of specimens injected with Indian ink (× 20). A, a string of ink protrudes from the renal pore area. B, opened renal canal; the hemal pore lies at the edge of the renal aperture.

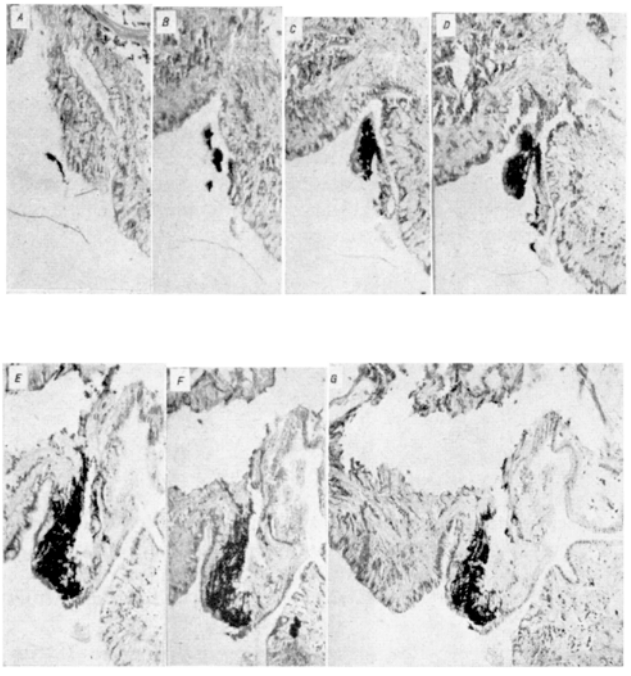

Fig. 2. Sections of the renal tube area (× 140). The ink can be followed from the lung cavity (A, B) via the hemal pore (C, D) to the spongious tissue slits (E, F), which communicate with the hemocoel (G). The renal opening and the renal canal can be seen in the right parts of C–G.

dissected. The tissue around the renal canal was black. However, the lumen of the renal duct was unstained, but, as illustrated by Figure 1A, a string of ink protruded from the renal pore area. When the renal duct was opened longitudinally it was found that the ink emerged from a very small opening located at the edge of the renal pore (Figure 1B). A microscopic study of a specimen injected with Indian ink showed, as can be seen in Figure 2, that this opening is connected via an extremely spongious system of narrow tissue slits with the hemolymph-filled sinuses in the lung wall. Figure 2 (C–F) demonstrates also that this opening is located adjacent to the renal pore in a common indentation of the lung wall.

In the sections, the ink can be seen only in the opening and in the spongious tissue, whereas the sinus is devoid of ink. This is most probably due to the fact that the blood circulation continued for some time during the narcotization period following the injection, so that the non-extruded part of the ink circulated all through the body and concentrated particularly around the kidney (Figure 1).

A final proof of the existence of the *hemal pore* was obtained when Indian ink was injected in blood sinuses of excised pieces of the renal tube area of the lung roof: the ink emerged from the pore.

It is concluded that *Lymnaea stagnalis* has the ability to discharge a large quantity of its hemocoelic blood via a special mechanism. The question of the physiological significance of this is answered, preliminarily, in the following way. Under ordinary conditions, much water continuously enters the body through the skin and the gut, and an equal quantity is eliminated by the kidney. To a large extent the shape of the head and foot is maintained by blood pressure, and during normal life these body parts are mostly extended. However, under sudden emergency conditions, for instance during the attack by a predator or when the snail meets unfavourable substances, the animal retracts very deeply into its shell and produces much mucus. It is clear that such heavy and often rapidly executed contractions are facilitated by the presence of an emergency outlet for the blood. In short, it is supposed that the hemal pore and perhaps also the tissue slits are normally closed, and are used only during violent body contractions.

Zusammenfassung. *Lymnaea stagnalis* verliert nach starker Reizung Blut aus einem der Nierenöffnung dicht anliegenden hämalen Porus, der in Verbindung mit dem System der Blutsinusse steht.

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Studies on a Compound Antagonistic to 5-Hydroxytryptamine

It is well established that 5-hydroxytryptamine (5-HT) induces foetal death in various phases of pregnancy[1–3] when administered to pregnant rodents. It is also known that various compounds with anti-enteraminic activity are able to inhibit this effect[1,3,4]. Just recently a new compound, 1-methyl-8β-carbobenzyloxy-aminomethyl-10α-ergoline (MCE), has been synthesized in our laboratories[5]. Its general pharmacological properties have been described extensively elsewhere[6,7].

The effect of anti-enteraminic active compounds upon the survival of foetuses whose mothers have been treated with 5-HT is of high interest. Some authors have identified the 5-HT or analogues as one of the factors involved in human toxaemia during pregnancy[8]. Stimulated by this hypothesis we have studied the activity of MCE in pregnant rats treated with 5-HT.