Endogenous Recovery from Colchicine-Induced Inhibition of Growth[1]

The mitotic inhibitor colchicine induces abnormal development in a great variety of plants and animals[2]. This substance causes the growing tip of roots to develop a tumefaction[3] at concentrations greater than the treshold value which produces its mutagenic effects in plant cells (Figure 1, left). In addition, the width of the coleoptile of wheat seedlings treated with colchicine is greatly increased (Figure 1, right) and its elongation is inhibited, giving rise to a globular-shaped organ[4]. These effects of colchicine originate from its ability to bind to subunit protein of the cell microtubules[5]. They might also be the consequence of colchicine forming a complex with particles of a proteinaceous nature[6] located inside the cell wall and distributed on the outer surface of the plasmalemma.

Exposure of wheat seedlings to high dose of the mitotic inhibitor – 1.0 mM – hinders irreversibly the elongation of the coleoptile. The study undertaken here shows, however, that this plant organ may overcome the inhitition after a certain period, despite the continuing influence of colchicine, when treated with a lower dose of the inhibitor.

Material and methods. Seeds of wheat (*Triticum aestivum* L. var. Rescue) were surfaced sterilized in a sodium hypochlorite solution, and washed in sterile distilled water. They were soaked 4 h in water, then germinated in petri dishes on moist filter paper in the dark at 24 °C. When the coleoptiles were 1 mm long, samples of 20 seedlings were transferred to containers with distilled water or an aqueous solution of colchicine. Incubation took place in the dark at 2 °C and 24 °C temperature. At the end of the incubation periods the coleoptiles were excised and their width and length measured.

Results and discussion. Figure 2 (left) shows that colchicine reduces the rate of coleoptile elongation. Nevertheless, the coleoptile recovers from this inhibition, and its growth rate approaches that of the control when the seedling is treated with 0.1 mM colchicine. On account of this, the phenomenon has been named 'endogenous regeneration'. This effect is best demonstrated by the experiments represented in Figure 2 (right). Incubation of wheat seedlings in a 0.1 mM solution of colchicine at low temperature (2 °C) induces asymmetrical oscillations during the course of the experiment. Despite the high value of the standard deviation, the drop of growth rate following the start of treatment, and the oscillatory recovery which takes place therafter, are events too great in magnitude to be attributed to random fluctuation.

The experiments also prove that it is not necessary to wash the colchicine solution out of the culture medium in order to induce regeneration of growth. This is in contrast to previous observations of reversal of the effect of colchicine[2] and of other spindle poisons such as vinblastine and griseofulvin[7]: e.g., the regeneration of the spindle in *Pectinaria gouldi* oocytes occurs only after the removal of the latter drugs.

The foregoing raises the question whether endogenous regeneration during a treatment with colchicine is due to the adaptive formation of an enzyme capable of decomposition of this substance. The finding that colchicine binds in vitro to DNA molecules[8], and prevents the cells from entering S phase[9], is an indication of the possible role of an induction mechanism in colchicine-regulated cell

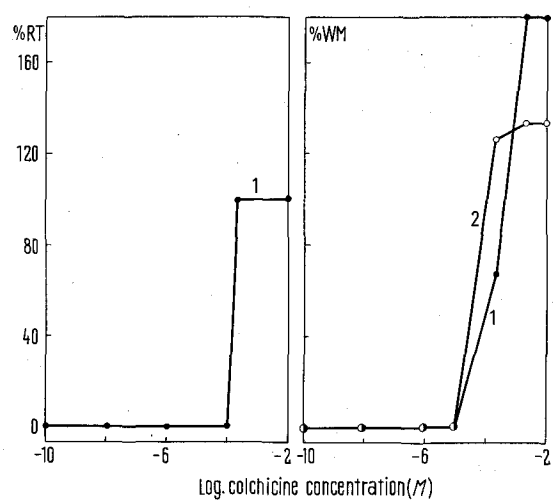

Fig. 1. Morphogenetic development of the root and the coleoptile of wheat seedlings as a function of the dose of colchicine received. Incubation took place in the dark at 24°C (1) and 2°C (2). Left: Root tumour incidence (% RT). The results are given as percentage of tumour incidence at the end of the incubation period (72 hours). Right: Maximum width of the coleoptile as percentage of the water control (% WM). The experimental points represent the average value (M) of 3 replicated experiments. Standard deviation $< M(14/100)$.

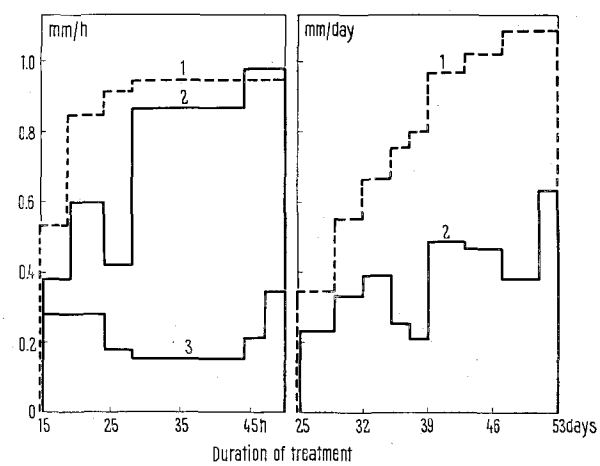

Fig. 2. Effect of concentration of colchicine on the rate of coleoptile elongation (mm/h; mm/day). Wheat seedlings were incubated in the dark at 24°C (Left) and 2°C (right) in distilled water (1) and in an aqueous solution of colchicine: 0.1 mM (2); 0.5 mM (3). The elongation rates shown are the average value (M) of 3 replicated experiments. Standard deviation $< M(32/100)$.

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development. Caution is warranted on the interpretation of this hypothesis. There is, however, a possible analogy of endogenous regeneration following a colchicine treatment with the growth recovery which occurs when the tip of the coleoptile of intact wheat seedlings is decapitated[10]. Elongation of the tipless coleoptile resumes, and this is the result of biochemical changes arising during a period of reduced growth activity. This shows again that induction may well operate during regeneration of growth in the wheat coleoptile.

The assumption discussed above is incorrect if it is proved that during regeneration colchicine controls cell division exclusively at the level of the mitotic apparatus. That is to say, through structural changes of the centromeres or the protein monomers of the spindle fibers, so that these cell sites would no longer be available for the colchicine activity. Another hypothesis is that this substance might be restrained from interferring with the cell metabolism in a way similar to streptomycin resistance in bacteria[11]: the latter drug induces a change in a protein sub-unit of the bacterial 30 S ribosome. Protein synthesis is insensitive thereafter to streptomycin inhibition. This would seem to show that endogenous regeneration following a treatment with colchicine might result from the structural modification of specific cell constituents which affects either the binding of the colchicine molecules or the functioning of their complexes.

Résumé. Des doses de colchicine inférieures au seuil de son activité mutagénique sont à l'origine de la variation périodique du taux de croissance in vivo de la coléoptile de blé. En particulier, le comportement oscillatoire de cette régénération endogène a lieu dans un milieu qui contient la colchicine pendant toute la durée de l'incubation.

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Blood Chemistry of *Lamellidens corrianus*

Lamellidens is one of the commonest Indian fresh water bivalves, but almost nothing is known about its body functions. Since blood reflects upon the over-all metabolism of animal tissues, a preliminary biochemical study of the blood of *L. corrianus* has been undertaken here as an initial step towards the understanding of the functional organisation of this mussel.

The alkaline (pH 7.9) blood of the mussel comprises amoebocytes and plasma, the latter constituting its major volume and being transparent and colourless.

Presence of a respiratory pigment could not be definitely established in the mussel blood. Spectrophotometry revealed an insignificant absorption in the visible spectrum range with faint indication of maxima at 530 nm and 560 nm, suggesting the vascular pigment, if present, to be more like haemoglobin than haemocyanin. But the benzidine test for haemoglobin and the potassium ferro- and ferricyanide test for iron gave negative results both with plasma and blood cells. However, treatment of proteins precipitated from 10 ml of pooled blood with potassium ferrocyanide produced an extremely light blue colour, indicating the presence of ferric ions in traces. It appears therefore, that even if an iron-containing vascular pigment is present, it is in such small amounts as to be of little use in the oxygen carrying needs of the mussel.

Plasma protein concentration of the mussel, estimated to be 0.9 g/l, is, like the concentration observed in other bivalves and gastropods[1], appreciably lower than that reported for cephalopods[1]. Since plasma proteins chiefly facilitate fluid movement across capillary end-loops, their high level in cephalopods and low level in other molluscs may be justified by the presence in the former and absence in the latter of capillaries between arteries and veins.

Glucose content of the mussel plasma was found to be 8.2 mg/100 ml. Martin[2] has suggested that it is wasteful for molluscs to maintain more than the minimal required blood glucose because of its imperative loss during filtration of fluid from the heart for urine formation. The presently observed glucose concentration, if considered as the minimum required, would imply a very low energy demand and hence an equally low over-all metabolism in the mussel, even in comparison with other bivalves whose reported blood glucose levels are considerably higher[2].

Amylase activity was studied in the plasma as well as in a homogenate of amoebocytes separated from 10 ml of pooled blood of the mussel. Plasma was found to hydrolyse 0.082 mg and the amoebocyte homogenate 0.015 mg starch/h/mg plasma protein. However, while the amylase activity of plasma must be non-functional, since blood cannot be the site of starch digestion, that of the wandering amoebocytes may be functional and indicative of a probable digestive role of these cells. Leakage of enzyme from blood cells is, therefore, a likely source of plasma amylase, but the much higher amylase content of plasma in comparison with that of blood cells would indicate leakage from other sources also.

Acid and alkaline phosphatase activities of the mussel plasma were respectively found to release 0.0107 and 0.0116 mg phenol per hour per mg plasma protein. These activities, being extremely low, do not appear to be functional. Moreover, the possibility of their leakage from amoebocytes to plasma cannot be ignored, specially since phosphatases have been histochemically demonstrated in the mussel amoebocytes[3].

Glutamic oxaloacetic transaminase activity of the mussel plasma was found to release 0.0187 mg pyruvate per mg plama protein. Presence of this enzyme in the plasma may be indicative of the existence of citric acid cycle in the mussel.

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