UNIVERSITY OF CAMBRIDGE

NATURAL SCIENCES TRIPOS Part IB

NATURAL SCIENCES TRIPOS Part II (General)

Thursday 5 June 2008 1.30 to 4.30

CELL AND DEVELOPMENTAL BIOLOGY (Practical Paper)

*Attempt **three** questions.*

*The answer to **each** question must be tied up separately with its own coversheet and with your **candidate number** and the **question number** written on it.*

*Also complete an **additional** coversheet with your **candidate number** and the numbers of **all** questions attempted.*

Write on one side of the paper only.

STATIONERY REQUIREMENTS

Metric graph paper

SPECIAL REQUIREMENTS

Calculator

You may not start to read the questions printed on the subsequent pages of this question paper until instructed that you may do so by the Invigilator

5. In the practical laboratory, you have tested the effects of different hormone combinations on xylogenesis in tissue discs from Jerusalem artichoke (*Helianthus tuberosum*). Sterile tissue discs were incubated in the dark on agar medium containing different concentrations of auxin and cytokinin. After 7 days, any increase in fresh weight of the tissue discs was measured and the tissue was stained with safranin O. The discs were examined under a microscope. The discs were then acid treated to dissociate the cells. A haemocytometer was used to count the number of stained xylem elements (wound vessel members) in a 0.1 microlitre volume, and then the total number of cells was estimated, using the measured volume of macerated cells. The number of differentiated xylem cells was normalised between samples by calculating the number of differentiated cells per gram fresh weight of tissue. In your experiment, you found:

Experiment 1

	control (no added hormones)	**auxin** (5.0 mg/L IAA)	**cytokinin** (0.1 mg/L zeatin)	**auxin** (5.0 mg/L IAA) **cytokinin** (0.1 mg/L zeatin)
Number of xylem cells per gram fresh weight	none	1.0×10^5	0.8×10^3	3.1×10^5
Fresh weight after 7 days	100%	410%	130%	315%

(a) What was the advantage of measuring the effects of the treatments in terms of fresh weight of tissue? What other parameters would be useful to know?

(b) From this data alone, what can you deduce about the effects of auxin and cytokinin on the cultured discs?

(c) Microscopic analysis showed that differentiated xylem cells had formed in a generally radial arrangement across the auxin-treated discs. Xylem cells were distributed as neatly spaced files through the tissue. What is the possible explanation for this?

(d) How could this type of spatial patterning arise from the application of a simple chemical like indoleacetic acid (IAA)?

A second experiment was designed in order to obtain more information about the roles of auxin and cytokinin in this process. Discs were cut mechanically to ensure a consistent size and weight. Each disc was estimated to contain around 20,000 cells when cut. The discs were then incubated for 7 days in the dark on nutrient agar with auxin and cytokinin supplements. At the end of the incubation, individual discs were stained and macerated, and the total number of cells, as well as the number of differentiated xylem cells, were estimated for each disc.

(please turn over)

Experiment 2

	control (no added hormones)	**auxin** (5.0 mg/L IAA)	**cytokinin** (0.1 mg/L zeatin)	**auxin** (5.0 mg/L IAA) + **cytokinin** (0.1 mg/L zeatin)
Number of xylem cells per disc	none	1.5×10^4	0.7×10^2	2.8×10^4
Total number of cells per disc	2.1×10^4	1.6×10^5	0.9×10^5	2.3×10^5

(e) Can you deduce any more information about the role of cytokinin from this second experiment?

(f) Tissue discs that had been cultured for 7 days on high levels of auxin were fragile, with irregular outgrowths of friable cells. Those cultured on cytokinin alone were smaller and mechanically stronger, with a more solid, dense appearance. What is the explanation for this?

In a third experiment, a DNA synthesis inhibitor was applied to tissue discs undergoing xylogenesis. The drug 5-fluorodeoxyuridine (FUdR) is an analogue of thymidine which inhibits DNA synthesis. Tissue discs were cut to a standard size (as above), and were incubated on nutrient agar plates with 5 mg/L indoleacetic acid in order to induce xylem cell differentiation. The plates were (i) untreated, or contained (ii) 1 µM FUdR, or (iii) 1 µM FUdR with 10 µM thymidine. The plates were incubated for 7 days in the dark, and the total and differentiated xylem cell numbers were measured for individual discs.

Experiment 3

	untreated	**1µM FUdR**	**1µM FUdR + 10µM thymidine**
Number of xylem cells per disc	2.5×10^4	1.3×10^2	2.1×10^4
Total number of cells per disc	2.2×10^5	2.5×10^4	1.9×10^5

(g) Describe the effects of FUdR and thymidine on the tissue discs.

(h) What do these experiments suggest about the role of cell division in xylem differentiation?