MORPHOGENETIC RESPONSES OF THE THALLUS OF *MARCHANTIA* TO SEVERAL GROWTH SUBSTANCES

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DURING recent investigations on the reactivity of young developing thalli of *Marchantia nepalensis* L. *et* L. to several growth substances we found that the different organs of the thallus responded differently to different growth substances.

Aseptic cultures were prepared from young thalli of about 1·0 cm. in length as inocula on a standard Knop's nutrient solution with the addition of three concentrations, namely, 1·0, 0·1 and 0·01 mg./l. of Indoleacetic acid (IAA), Indolebutyric acid (IBA), Indolepropionic acid (IPA), α-Naphthaleneacetic acid (NAA), Naphthoxyacetic acid (NOA), 2, 4-Dichlorophenoxyacetic acid (2, 4-D), Maleic hydrazide (MH), 2 : 4 : 5 : Trichlorophenoxyacetic acid (TCPA), 2 : 3 : 5 : Triiodobenzoic acid (TIBA), and 2, 4-Dinitrophenol (2, 4-DNP). Both solid and liquid cultures were grown under artificial light from fluorescent tubes (Natural) giving 2,600–3,000 lux of 17 hours duration in 24 hours cycle at the level of the cultures. The temperature was regulated at 24°-25° C.

The most striking effects may be summarized as follows :

(*a*) Highest concentration (1·0 mg./l.) of NOA, 2, 4-D, TCPA, IPA, IAA and IBA and 0·1 mg./l. of NAA stimulated rhizoid formation and inhibited thallus growth but NAA in its highest concn. was inhibitory to rhizoid formation. Furthermore NAA, NOA and TCPA induced rhizoid formation not only on ventral but also on dorsal surfaces of thalli extending up to their apical regions. The latter substances including 2, 4-D in their lowest concn. (0·01 mg./l.) was even inhibitory to thallus growth.

Highest concn. of MH, TIBA and 2, 4-DNP neither stimulated nor inhibited rhizoid formation and thallus growth and even their lower concns. (0·1 and 0·01 mg./l.) were not stimulatory to either rhizoid formation or to thallus growth.

(*b*) Highest concn. of NAA, NOA, TCPA and 2, 4-D produced globular masses of callus-like tissue on inoculated thalli. The globular masses were mostly yeliowish-brown or brown in colour except those produced by TCPA which were green. Even at a later period of growth the globular masses did not differentiate into thalli but they did so on being transferred to control media. However, these differentiated thalli did not attain normal size (Fig. 1 A).

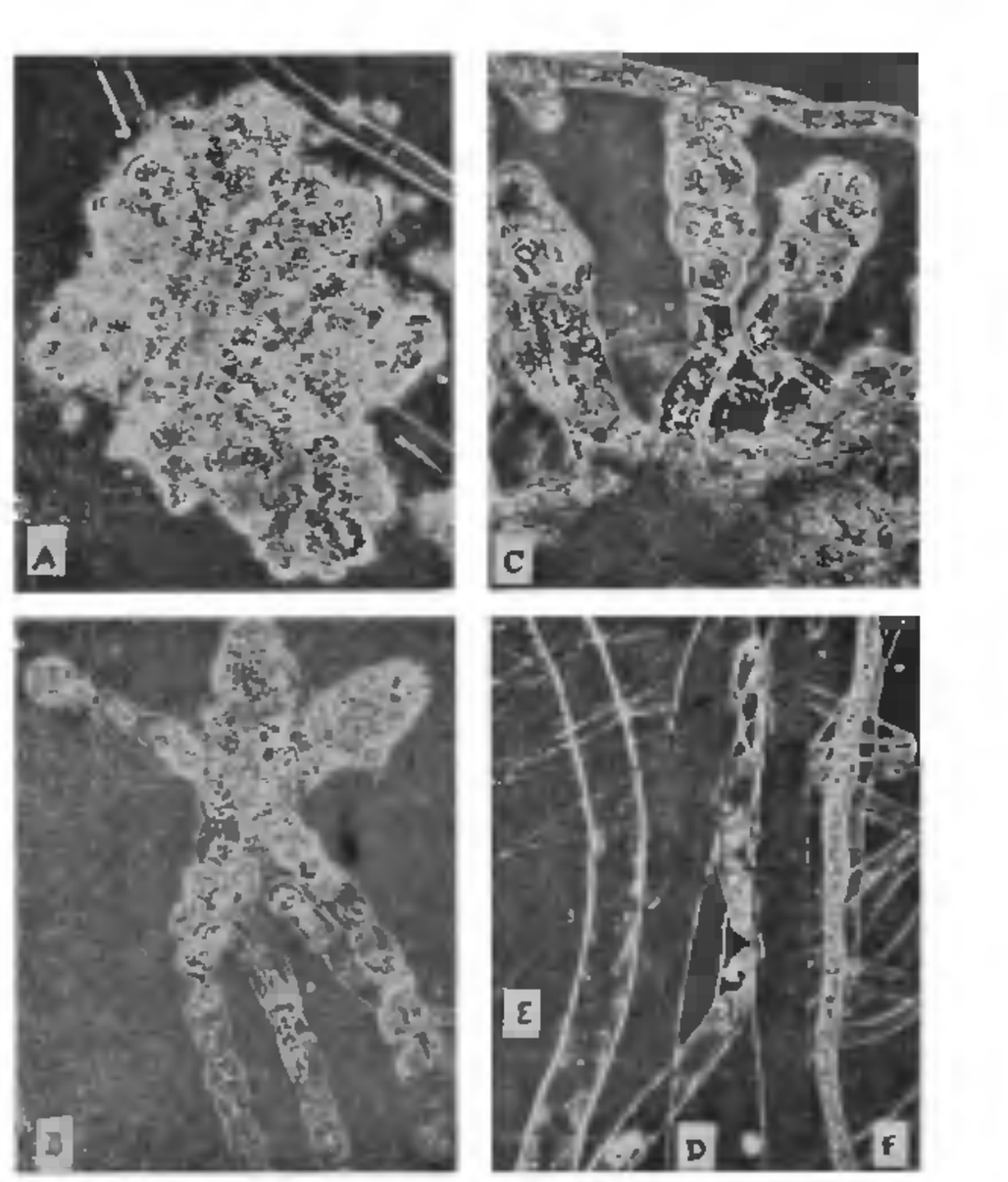

FIG. 1. *Marchantia nepalensis.* A, Callus like growth, × 75; B *and* C, Filamentous growth terminated by differentiating thalli—B × 55, C, × 90; D A tuberculated rhizoid (× 140) showing pegs in its distal region but not in its terminal portion; E, Tip region of the tuberculated rhizoid shown in D, × 140; F, a tuberculated rhizoid (× 50) showing prominent pegs.

(*c*) Highest concn. of IAA, IBA, IPA, TIBA and 2, 4-DNP produced globular masses of callus-like tissue on inoculated thalli at an earlier period of growth but at a later period these masses differentiated into new thalli. Whereas highest concn. of MH and TIBA produced new thalli directly from inoculated thalli.

Commonly new thalli were minute and brittle in liquid cultures..

(*d*) New thalli developed in three ways : (*i*) directly from cells of inoculated thalli commonly from their distal regions ; (*ii*) from cells of globular masses ; and (*iii*) from filamentous growth of cells of inoculated thalli and of globular masses (Fig. 1 B, C).

(*e*) All the concns. of IPA, TIBA, 2, 4-D and 2, 4-DNP inhibited typical tuberculate rhizoid formation in their *liquid* but not in their *solid* cultures ; but only in one case, that of 1·0 mg./l.

NAA, tuberculate rhizoids were not formed in liquid as well as in solid cultures.

Pegs of tuberculate rhizoids were poorly developed in liquid cultures containing 1·0 and 0·1 mg./l. of TCPA, IAA, NOA and IBA and even in control liquid cultures (Fig. 1 D, E. F).

(*f*) Highest concn. of NAA, NOA, TCPA and 2, 4-D and 0·1 mg./l. TCPA inhibited gemma-cup formation in solid as well as in their liquid cultures.

Highest concn. of IPA, IBA and TIBA and 0·1 mg./l. NOA inhibited gemma-cup formation in their solid cultures only. In solid cultures gemma-cups were formed mostly in submerged thalli but very rarely in aerial ones.

Gemma-cup formation was markedly pronounced especially in liquid cultures containing 2, 4-DNP and TIBA.

(*g*) Germinated gemmæ with smooth rhizoids were found within gemma-cups in cultures containing MH, NAA, NOA, 2, 4-D and 2, 4-DNP and also in control cultures.

Germinated gemmæ without rhizoids were found within gemma-cups in cultures containing MH, NAA, NOA, 2, 4-D and 2, 4-DNP and also in control cultures.

It is evident from the observations mentioned above that rhizoids and thalli of *Marchantia nepalensis* like roots and shoot-buds of higher plants responded in a similar way to stimulatory and inhibitory effects of higher and lower concns. of auxins and antiauxins used. It may thus be envisaged that there exists a basic biochemical pattern common to roots and shoot-buds of higher plants and rhizoids and thalli of *N. nepalensis* indicating homologies in organization. In higher plants this basic biochemical pattern has further been elaborated during evolution giving rise to more complex organizations like roots and shoot-buds.

The antiauxins like MH, TIBA and 2, 4-DNP are well known for their peculiar effects in higher plants but they have not produced any such effects in the highest concn. used in this experiment. It is to be seen whether they can produce any morphogenetic response in still higher concns.

Allsopp[1] obtained callus-like tissue in *Fossombronia pusilla* (L.) Dunn. and *Reboulia hemispherica* (L.) Raddi. in cultures containing glucose. In the present experiment globular masses produced by certain auxins and antiauxins mentioned previously are similar undifferentiated callus-like tissue which begins to differentiate when transferred to nutrient media without the growth substances. According to Bünning[2,3] polarity is of decisive importance for all the processes of differentiation, and suppression of polarity by direct effects of physical or chemical factors on the protoplasm, will prevent normal differentiation but may allow cell division to continue. On the basis of this hypothesis, the action of these growth substances on young developing thalli could be due to their direct effect on the internal gradients of chemicals.

Regeneration of thalli directly from cells of inoculated thalli or from filamentous growth of cells of inoculated thalli or of globular masses throws light on the debated problem of the existence of hepatics. Various lengths of filamentous growth observed in the present experiment prior to the differentiation of a normal thallus from a two-sided apical cell indicate that the filamentous growth results from induction of successive transverse divisions in the thallus initial by different growth substances for a variable duration. Similar prolongation of thallus initials into filamentous growth prior to differentiation by a physical factor like unilateral or weak illumination has been reported by Goebel,[4] Pande,[5] Mehra and Kachroo[6] and others in other hepatics. This filamentous growth is not comparable to protonemal growth of mosses where it is a distinct and a constant phase of the gametophyte. These experimental evidences support Campbell's[7] conclusion on this issue.

Inhibition of *tuberculate* rhizoids and not of *smooth* ones by certain growth substances and the formation of tuberculate rhizoids with various degrees of poorly developed pegs in cultures containing certain growth substances bring out that the two types of rhizoids are not only morphologically but also physiologically different. This point is emphasized by the occurrence in cultures of certain tuberculate rhizoids whose distal regions are with poorly developed pegs but their terminal regions are without them. Furthermore the presence of poorly developed pegs in tuberculate rhizoids of *control liquid* and not of *control solid* cultures indicates that the hydration of the colloidal constituents of the cell may have affected synthesis of peg-substance which seems to be pecto-cellulose on micro-chemical tests. Synthesis of peg-substance was completely inhibited in *liquid* but not in *solid* cultures containing certain growth substances as mentioned previously. This finding tends to help in the understanding of the speculated functions of the two types of rhizoids of the Marchantiales. We are also well aware of the morphogenetic effects of water in higher plants as well expressed by the phenomenon of heterophylly and the production of

land and water forms of certain amphibious plants.

LaRue and Narayanaswami[8] state that the gemmæ of liverworts do not germinate within gemma-cups unless they have been detached from the parent body. Audus[9] suggests that "in this the controlling agent may be a specific inhibitor produced by the parent tissue". In the present experimental conditions germinated gemmæ with or without rhizoids have been found within gemma-cups in control cultures as well as in cultures containing certain growth substances. It is also interesting to note that certain growth substances have inhibited the formation of gemma-cups. The findings also indicate that production of gemma-cups is conditioned by good humid conditions.

These observations will be considered in greater detail elsewhere.

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RESEARCH INTO BOILER CIRCULATION THEORY

A REPORT on an extensive series of experimental investigations of the fundamental factors influencing the circulation process in high pressure boilers was presented to a meeting of the Institution of Mechanical Engineers in London, on 29th March 1961, by Haywood, Knights, Middleton and Thom.

The research project was sponsored by the Water-Tube Boilermakers' Association and was carried out by the authors at Cambridge. The primary object of the research was to establish experimental data relating to the flow conditions and pressure drop of high pressure steam-water mixtures flowing along heated and unheated pipes—in both horizontal and vertical positions. Simple boiler circulation theory is based on the assumption that the steam-water mixture moves as a homogeneous fluid, but there was little existing data on effects of relative velocity between the steam and water phases, a phenomenon which was known to exist under actual flow conditions in a boiler circuit. The paper describes an isotopic technique of determining this relative velocity of the two phases.

This consists in measuring the absorption of gamma-rays in their passage through the two-phase mixture at the outlet from the test section. The results from the gamma-ray equipment—in which the beam was provided by a radioactive isotope of cæsium—enabled calculations to be made of the apparent density of the fluid mixture, and consequently the respective velocities of the two phases at that point. Preliminary tests involving scans along a number of chords of the tube cross-section showed that the density distribution of the fluid was different with horizontal and vertical pipes. The data thus obtained from these and other tests were used to calculate slip correction factors, by means of which the acceleration and gravitational pressure drops—calculated according to homogeneous theory—could be corrected for the effects of slip. The paper presents an analysis of the pressure drop measurements made on the 1-inch and 1½-inch bore pipes in the vertical and horizontal positions, with particular attention to the frictional pressure drops in the horizontal pipes.

In their conclusions the authors state that, over the range of variables covered in the tests, the experimental pressure drops at 2,100 p.s.i. abs. are close to the values predicted by homogeneous theory for both horizontal and vertical pipes. For horizontal pipes at the lower pressure, homogeneous theory gives closer prediction of the pressure drop for heated than for unheated pipes, owing to the opposing effects of two-phase flow conditions on the frictional and acceleration pressure drops in the heated pipes. For vertical pipes at the lower pressures, the experimental pressure drops are significantly greater than the values predicted by homogeneous theory. The gravitational contribution to the total pressure drop is dominant, and it is influenced significantly by the effects of slip.

The results of the work have provided a wealth of information in a field in which there has been previously a scarcity of reliable data. —(*Courtesy*: The water-Tube Boilermakers' Association, 8 Waterloo Place, London, S.W. 1).