source of cuticular water may also account for the fact that the water activity of the cuticle of terrestrial isopods is higher than that of the blood[6].

What mechanisms regulate the opening of the mouth and the anus is unknown yet, but there are some clues. If the fourth pair of legs is removed the rate of water loss from the body increases greatly in dry air[7]. A removal of a pair of legs interrupts the capillary system, and a drop of fluid placed on one end of the body will not flow over the amputated site. Hence it looks like the other end of the body 'dries out', which subsequently results in a higher output of fluid to compensate for it. What the actual feed-back mechanisms are can only be speculated. For instance, superficial water receptors with intestinal volume receptors might be involved. It is a curious fact that the occlusion of either the mouth or the anus alone affected the evaporation pattern, which refers to the possibility that both ends of the alimentary canal are regulated by a same feed-back system. The wall of the intestine may be the site where blood concentration and content is regulated (cf. HOROWITZ[8]). At the light microscope level GUPTA[9] found in both *P. scaber* and *Oniscus asellus* rectal glands which correspond to those occurring in many insects, and their main function could be regulation of the body water. The presence of a hormonal system regulating the output of water in terrestrial isopods has been suggested[1]; the actual site of action of this system may therefore be the wall of the intestine. Also, a major part of the nitrogenous waste in terrestrial isopods occurs in the form of gaseous ammonia[10]. It is conceivable that at least part of this ammonia is released in the intestinal fluid. Hence the discharge could also be regulated by a gradual build-up of ammonia in the intestine.

The problem concerning the presence of a waterproofing barrier in the epicuticle of terrestrial isopods has remained unresolved (cf. EDNEY[2]). Apparently, the presence of external water on the cuticle has brought about a bias in evaporation rates. Furthermore, the term 'critical temperature' at which the permeability of the cuticle abruptly increases[11] needs some re-evaluation in the light of the present theory on intestinal discharge at least as far as the terrestrial isopods are concerned. Namely, a high temperature treatment may also affect the hormonal system that controls the output of water; the result may be a release of a diuretic factor and an increase in the rate of fluid discharge. A possibility remains that this may apply to both isopods and insects, especially in view of the experiments on the role of certain insecticides in an induction of diuresis[12,13].

Zusammenfassung. Neue Vorstellung über den Evaporationsmechanismus des Wassers bei terrestrischen Isopoden mit Regulation des Flüssigkeitsabflusses durch Mund und Anus. Die Flüssigkeit aus dem Darm wird durch das ventrale Wasserleitungssystem abgeleitet und über den Körper verteilt.

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[6] O. V. LINDQVIST, I. SALMINEN and P. W. WINSTON, J. exp. Biol., in press.
[7] O. V. LINDQVIST and H. MÄKILA, in preparation.
[8] M. HOROWITZ, Entomologia exp. appl. *13*, 173 (1970).
[9] M. GUPTA, Nature *191*, 406 (1961).
[10] W. WIESER and G. SCHWEIZER, J. exp. Biol. *52*, 267 (1970).
[11] J. W. L. BEAMENT, J. exp. Biol. *35*, 494 (1958).
[12] F. MORIARTY, Biol. Rev. *44*, 321 (1969).
[13] S. H. P. MADDRELL and J. E. CASIDA, Nature, Lond. *231*, 55 (1971).
[14] This work has been supported by grants from the Research Council of the University of Dayton.

Callusing and Regeneration of Cell-Aggregates and Free-Cells of a Hepatic: *Asterella angusta* Aust.

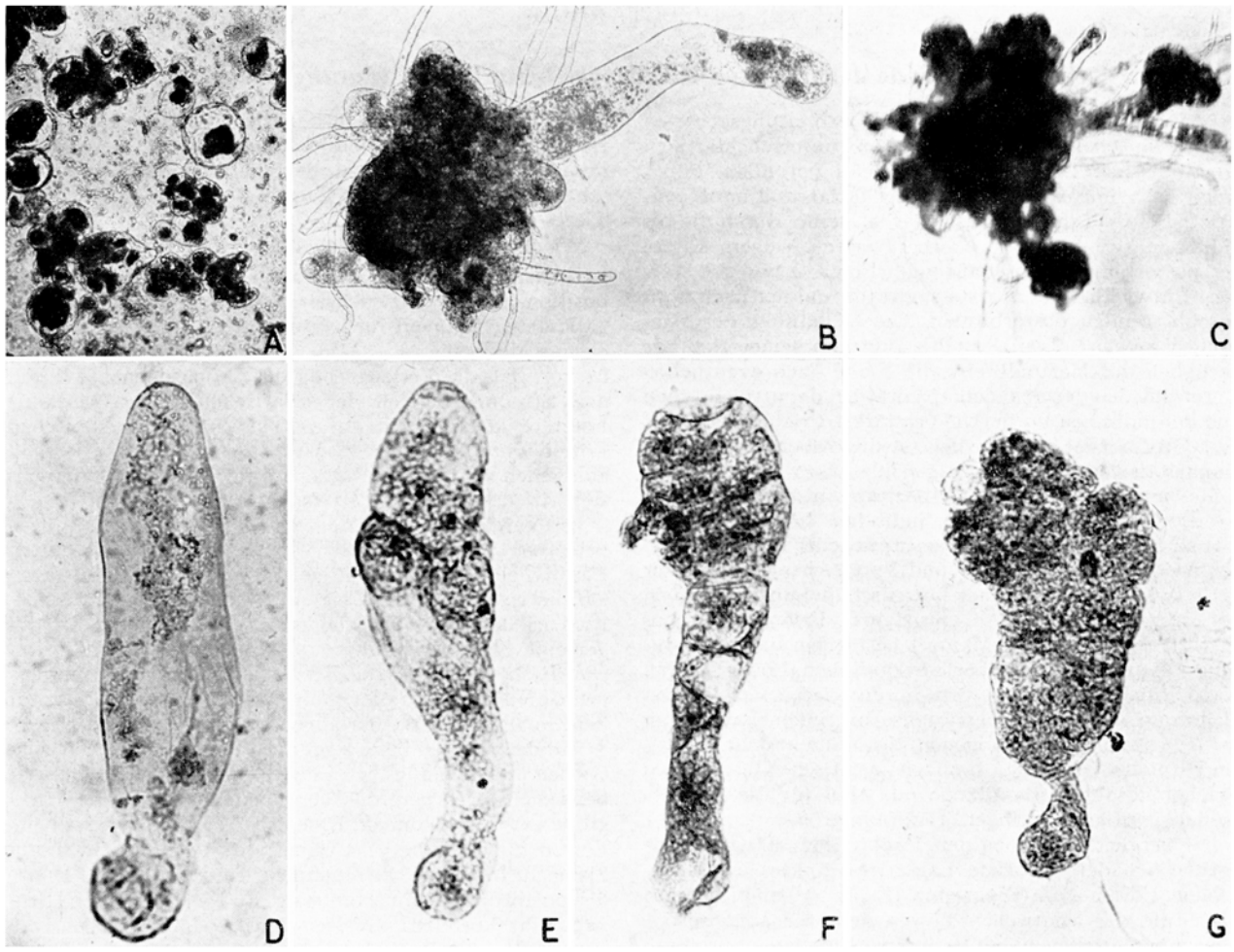
There are only a few instances of callus formation in bryophytes[1,2]; and these lack detailed information about the process. In the present investigation, methods to raise callus cultures, and to see whether somatic cells can be made to simulate spores, were attempted.

Material and methods. Spores of *Asterella angusta*, a common Himalayan hepatic, were aseptically sown on basal medium (BM) comprising Knop's mineral salts (half-strength), trace elements (1 ppm, after NITSCH), ferric citrate (10 ppm) and 2% sucrose. The spores germinated only in a few cultures after 2 weeks. Under high light intensity (3000 lux) the germ-tube was short and soon a thallus organized at its apex, whereas under 20–25 lux the spores formed long germ-tubes and the development of thallus was delayed. One of the thalli was propagated vegetatively and its progeny used for all experiments. To induce callus individual thalli from BM were implanted to different media. For obtaining free-cells small pieces of calli were transferred to 5 ml of liquid medium in Tumble (T) tubes and put on a continuous rotary shaker (3 *rpm*). The cultures were maintained under controlled conditions of light (10–12 h in a 24 h photocycle), temperature (25 $\pm$ 2 °C) and relative humidity (50–60%). Experiments were repeated once with a total of 24 cultures per treatment.

Results. On medium with increased sucrose (3%) the young regenerants showed callusing in a few cultures. A further increase (4%) favoured regeneration, but the regenerants remained stunted and at the end of 6 weeks callusing was observed in 12% cultures. With 6% sucrose there was profuse regeneration and the regenerants soon callused. However, the tissue formed was slow growing and started differentiating in situ. To find out whether it was osmotic effect, mannitol was added. On BM+1% mannitol regeneration was poor and in isolated instances the regenerants formed callus. With an increase in mannitol, the percentage of cultures showing callusing increased and on medium with 4% mannitol regeneration was inhibited, and instead the explants callused from the apical end posteriorly. A transfer of callus to BM induced differentiation. After 3–4 days numerous germ-filaments developed from the callus surface. Under low light (20–25 lux) the filaments were long (2054 µm), narrow with fewer chloroplasts concentrated towards the apex. Under 3000 lux the average length of the filaments was only 206 µm, they were broad, and contained numerous chloroplasts. In these cultures thalli appeared within a week. Thus numerous thalli could be obtained from a callus mass.

In shake cultures, after 3 days a suspension of small cell-groups and free-cells resulted (Figure A). In mannitol medium the cells failed to elongate, whereas in the BM the

[1] D. E. MEYER, Naturwissenschaften *40*, 297 (1953).
[2] A. ALLSOPP, Nature, Lond. *179*, 681 (1957).



Callusing and regeneration in *Asterella augusta*. A) Free-cells and small cell-groups in suspension × 57.8 B, C) The cell-groups having formed germ-filaments and thalli × 86,7, 49.3. D) Formation of germ-filament from free-cell × 178.5. E, F and G) Stages in the development of thalli from free cells × 178.5.

cells developed germ-filaments of variable length within a week and after 2–3 weeks thalli were formed (Figures B and C). Free-cells also regenerated to form germ-filaments and thalli developed at their apices. In the next experiment to ascertain the regeneration of free-cells, the supernatant from shake cultures, containing mostly free-cells, was decanted and a few drops of it were pippeted to flasks containing 5 ml of liquid BM and also plated on the agar surface (BM) in petri plates. In these cultures individual cells formed germ-filaments and gave rise to thalli (Figures D–G).

Discussion. The factors inducing dedifferentiation are little understood. Masses of apolar cells may result from suppression of polarity caused by various treatments to the protoplasm[4], or auxin-cytokinin system largely influences dedifferentiation[5].

In the present investigation, a mere increase in sucrose induced callusing, but the tissue formed was unstable. With the addition of mannitol stable tissue was obtained, thereby indicating that the callusing might have been brought about by an increase in osmotic potential. Increased sucrose could not induce a stable callus; probably it was readily metabolized, but mannitol was effective.

The tissue differentiated only on the basal medium. In the present system cell elongation is the prerequisite for differentiation. Mannitol prevents cell elongation[6] and consequently inhibits differentiation. Free-cells regenerated directly unlike that of *Polytrichum commune* which formed cell-aggregates[7]. In the process of regeneration, sporeling pattern was simulated. The system is well suited for studies on the factors controlling the initiation of thalli in hepatics[8].

Zusammenfassung. Nachweis, dass im Lebermoos *Asterella angusta* erhöhte Saccharose-Konzentration Kallusgewebe induziert, welches sich in der Folge wieder ausdifferenziert, während Mannitzugabe zu stabilem Kallusgewebe führt.

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[3] M. Ward, Science *132*, 1401 (1960).
[4] E. Bünning and D. V. Wettstein, Naturwissenschaften *40*, 147 (1953).
[5] R. J. Gautheret, *Cell Differentiation and Morphogenesis* (Elsevier, Amsterdam 1966), p. 55
[6] R. Cleland, Ann. N.Y. Acad. Sci. *144*, 3 (1967).
[7] M. Ward, Nature, Lond. *204*, 400 (1964).
[8] Acknowledgments: I am grateful to Professor B. M. Johri and Dr R. N. Chopra for encouragement, to Professor P. R. Bell (University College, London) and Dr P. S. Ganapathy for discussions.