agreement with our counts of 19 ($n$) and 38 ($2n$) for *B. floridae* than those reported by the earler workers for B. *lanceolatum*. The sizes of the chromosomes reported for the Japanese lancelet are similar to those in *B. floridae*. However, Nogusa[6,7] reported that the chromosomes in *B. belcheri* are rod-shaped, indicating subterminal to terminal centromeres. He did not mention any median to submedian centromeres as found by us in *B. floridae*. In addition, he found a remarkable size difference in 2 chromosomes which he interpreted as being an $XY$-pair of sex chromosomes. We did not see any such chromosomes in *B. floridae*.

There is much uncertainty regarding the phylogenetic position of lower chordates of the subphyla Cephalochordata (lancelets) and Urochordata (tunicates). In addition, there has been much speculation on the relationships of these lower chordates to the most primitive of vertebrates, the agnathans. Recent studies on the chromosomes of tunicates[11] and the relative cellular DNA content of tunicates, lancelets, and agnathans[12], have given us a better understanding of relationships among these groups. The diploid chromosome numbers of 32 and 38 for *B. lanceolatum* and *B. floridae* are very close to those of 28 and 32 reported for two species of tunicates[11]. In addition, the size of the tunicate chromosomes[11] was less than 3µm as in *Branchiostoma*. Furthermore, relative DNA values of the lancelet and tunicate were lower than the lowest value obtained for any vertebrate and seem to indicate a somewhat close lancelet-tunicate relationship[12].

A comparison of lancelet and lamprey chromosomes indicates that their only similarity seems to be in their small size. Lamprey chromosomes are generally under 3µm in length[10,13–16], except in Australian lampreys, whose chromosomes are up to 6 µm in length[13,14]. The very dissimilar diploid chromosome numbers ranging from 70 to 168 in lampreys[16] easily distinguish them from lancelets.

The hagfishes with diploid chromosome numbers of 48 and 52 are closer numberwise to lancelets than are lampreys; however, hagfish chromosomes are generally larger, some being up to 5 µm in length[11].

A comparison of the DNA values relative to the human leucocyte value has shown that the lancelet with 17% is closer to the lamprey with 38% than to the hagfish with 78%[12].

Our cytological findings of a small diploid number and small-sized chromosomes in *B. floridae* are consistent with the idea that vertebrates evolved from an organism with a very small amount of DNA, and that an increase in DNA by gene duplications and polyploidization began to occur before the jawed fishes evolved[12].

*Résumé.* Une étude des chromosomes de *Branchiostoma floridae* Hubbs, une espèce de lancelet de l'Atlantique occidentale, révéla la présence d'haplocaryons et diplocaryons au nombre de 19 et de 38 respectivement. Ces chromosomes furent comparés à ceux de 2 espèces de lancelets du Vieux Monde, *Branchiostoma lanceolatum* et *B. belcheri*. On résume les études concernant les chromosomes des Urochordata et des Agnatha et on émet des hypothèses sur las relations possibles entre les Protochordata et Agnatha.

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## Evaporation in Terrestrial Isopods is Determined by Oral and Anal Discharge

The evaporation rates of the terrestrial isopods *Porcellio scaber* Latr. and *Armadillidium vulgare* (Latr.) are known to vary cyclically in dry air[1]. The average length of time from one maximum to the next was found to be about 1 h. This cycle was also shown to affect the orientation of these animals in a humidity gradient[1]. I have repeated some of these evaporation experiments using a finer technique, and as a result I now introduce a new theory concerning the regulation of water loss in terrestrial isopods. In short, the evaporation cycle appears to be the result of an oral and anal discharge of fluid; this fluid then spreads over the cuticle and contributes to the overall rate of evaporation of water from the body. One implication of this is that the water permeability data published (cf. reviews by Edney[2] and Lindqvist[1]) may be too high.

The weight changes of *Porcellio scaber* were measured using a Cahn RG Electrobalance connected to a 1 mV recorder. The readings were made to the nearest 0.01 mg. The animal was kept in a small brass cage on the stirrup. The humidity of the weighing chamber was controlled by Drierite (for 5 ± 2% relative humidity, RH) or a saturated solution of $KH_2PO_4$ (for 95 ± 1% RH). Representative curves for the water loss are given in the Figure. It was confirmed that the rate of water loss at both low and high humidity varies cyclically, though the period length, especially at the beginning, appeared to be highly variable. There were stepwise changes in the rate of water loss; these steps were sharp and generally occurred in 1 to 3 min. On the other hand, there were also long-lasting changes in the rates of water loss; a half an hour or a shorter period of relatively low rate was followed by a sharp change to a relatively higher rate, and so on. Measurements for each animal were made for 3.5 h and towards the end of this period the changes in rates leveled off and occurred at longer intervals. In principle, the evaporation patterns of *A. vulgare* were similar to those of *P. scaber*.

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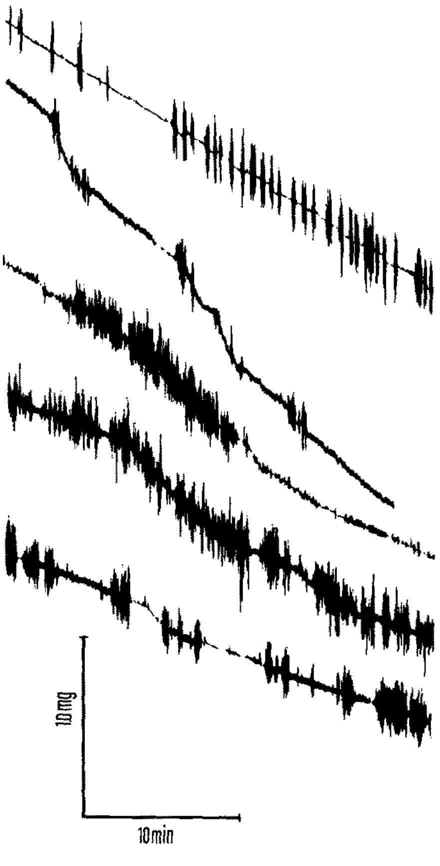
This evaporation pattern prompted me to think of some other mechanisms for the regulation of water loss than mere changes in the cuticular permeability (cf. Lindqvist[1]). Subsequently, occlusion of the anus by sealing the last 3 pairs of pleopods with paraffin greatly diminished or totally abolished the changes in the rate of water loss. Among the 12 *P. scaber* whose anus was blocked, 4 showed a single conspicuous stepwise change during the course of 3.5 h, but on examination the paraffin block was also found to have become loose. Blocking the anus proved difficult, though the observed bursts of increased rates may have also been caused by oral discharge. Sham-operated animals with a paraffin block on the mid-ventral side resembled intact animals in their water loss patterns. The mouth of 8 animals was similarly blocked and as a result the abrupt changes were also damped or nearly abolished, which proves that the mouth may also contribute to the discharge of fluid from the alimentary canal. Blocking the mouth and the anus at the same time abolished all of the stepwise changes. Among the 12 intact animals there was one which lacked the changes in water loss; this individual showed no locomotor activity at all during the test period. The higher rates of water loss were often, though not invariably, associated with bursts of locomotor activity. The cage was so small, however, that only the search movements do show up, and the animal was unable to do any running activity under these conditions.



Examples of curves showing the weight changes of 5 adult *Porcellio scaber* males after 60 min desiccation in dry air (about 5% RH) at 24 °C. The uppermost curve refers to an individual whose mouth has been blocked with paraffin. Note that there is great individual variation in evaporation rates. The up and down movements of the recording pen indicate locomotor activity.

On the ventral side of terrestrial isopods there are 2 lateral capillary channels[3,4]. When a drop of fluid is placed in either the anterior or the posterior end of a channel, the fluid will appear on the opposite end of the body. Verhoeff[3] supposed that free water from the substrate is collected with the aid of the uropods, from where the water is then carried into the anterior direction to moisten the pleopods. However, it seems that these capillary channels are also used to bring fluid from the mouth and/or the anus all over the body: a drop of methylene blue solution placed on the mouth will appear in about 10 sec all along the lateral channels and also between the dorsal tergites. From these channels the dye then gradually spreads laterally and mid-ventrally. The rate of water movement was slow if the animal did not move (e.g., showed death feigning) in which case it took over a minute for the dye to reach the opposite end of the body. A slight mechanical irritation of the anal or the oral region will often cause a massive discharge of fluid which really can be seen spreading through the ventral channels. We may note here that Verhoeff[3,4] already suggested that the rectum and the mouth might be employed in picking up water from the capillary system.

The pleopods of terrestrial isopods are normally covered with a thin film of fluid, whose most probable origin is the capillary system itself. Evaporation of this particular water from the pleopods may account for the rapid initial rate of water loss in both intact and occluded individuals (cf. Lindqvist[1]; Bursell[5]). The 6 specimens whose anus, pleopods and mouth were covered with paraffin failed to show any initial peak in water loss but the rate remained steady from the very beginning throughout the 3.5-h desiccation period. After the free water surrounding the pleopods and the rest of the body is lost, new fluid may be discharged at intervals from the mouth and/or rectum to replace it. The higher rate of water loss that was often found associated with searching activity may result from a higher rate of spreading (or discharge) of the fluid through the water channel system. Also, fanning movements of the pleopods may be involved. On the other hand, a moving animal in still air may have a somewhat higher rate of water loss due to a disturbance of the humidity gradient around it; this effect, however, may be negligible, since Bursell[5] reported that the rate of water loss in terrestrial isopods is related to the saturation deficiency at high humidities, but below about 70% RH the rate appeared rather steady and independent of the humidity of the air. The factors contributing to the pattern in evaporation may be complicated since earlier it has been observed that there is an inverse relationship between rate of water loss and the running locomotor activity, although this was based on an indirect evidence[1]. The main reason for this relationship in the context of the present theory could be that there is no discharge of fluid during intensive locomotor activity.

The presence of fluid from the gut on the cuticle could also explain some of the data published on the cuticular water content[6]. Namely, the water content of the cuticle on both *P. scaber* and *A. vulgare* remains unchanged during desiccation in dry air; it was supposed that this water enters the cuticle directly form the haemolymph. However, the stability of the cuticular water and its relative independency of evaporation rates may be explained better if the source of water is external. This

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[4] K. W. Verhoeff, Z. wiss. Zool. *118*, 365 (1920).

[5] E. Bursell, J. exp. Biol. *32*, 238 (1955).

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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## 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 ± 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

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