as controls. A week after the death of all females, the larvae present in the medium were scored. In 3 of the 5 replicates in treatment, there were no larvae and in the remaining ones their number was much less compared to controls (Table).

Since an unmated female is always infertile, the absence of larvae in 3 replicates suggests that the males had failed to mate even though the females were in the vicinity. It is therefore reasonable to infer that failure to mate was probably due to exhaustion resulting from excitation during previous exposure to the pheromone. A few matings discernible in the other 2 replicates suggest that either some of the males were not exhausted, or they recovered in due course.

Mating ability of *T. granarium* males previously exposed to female sex pheromone

Replicate	No. Larvae	
	Treatment	Control
1	0	280
2	135	308
3	0	207
4	39	234
5	0	208

Résumé. L'exposition prolongée des *Trogoderma* mâles vierges au phéromone sexuel femelle a réduit considérablement leur capacité d'accouplement, quoiqu'il y eût des femelles à proximité. Ce fait résulte probablement de l'épuisement des organes excités pendant l'exposition au phéromone sexuel.

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PRO EXPERIMENTIS

Autoradiography of Whole-Mounts of Isolated Ectoderm of the Body Column of *Hydra*. A New Method[1]

So far studies concerned with cell migration, cellular turn-over, and structural aspects of axial gradients in *Hydra* have been performed according to the classical histological techniques of using serial sections. Besides being time consuming, this procedure does not exclude erroneous quantitative estimations, since the possibility of counting the same cell twice on neighbouring sections cannot be avoided, especially in the case of radioactive labelling.

The method proposed serves the same purposes, but since it works with whole-mounts of the entire ectoderm of the body column, the cellular situation does not need to be reconstructed. Cells and groups of cells appear in their natural and undisturbed two-dimensional configuration. In isolating the ectoderm from the endoderm, we have improved and refined the technique described earlier[2–4] and have made it available for autoradiographic studies.

The experimental animals, *Hydra attenuata* Pall.[5], were grown in artificial culture medium[6]. The hypostome and the tentacles of the polyps were amputated just below the tentacle crown. The remaining body was transferred to a slide previously coated with a thin film of egg albumin. In order to split open the tubular body of the *Hydra*, the tip of a watch maker's forceps was pushed longitudinally through the gastral cavity. The body wall was then lengthwise sliced open by rubbing the loop-like tip of a wolfram needle against the edge of the tip of the forceps. The opened body wall was then laid out on the surface of the slide, so that the ectodermal layer was in direct contact with the slide surface, while the endoderm faced upward.

The split open body was anaesthetized by adding a drop of MS 222 (0.02 g/10 cm³) and Novocain (0.5 g/10 cm³) previously mixed in a proportion of 5:1. 10 minutes afterwards the body wall was completely anaesthetized and had reached its maximal degree of two-dimensional extension (various other drugs tested failed to give satisfactory results, because the body wall, instead of expanding, remained contracted or shrank when alcohol and acetic acid were later added to separate the endoderm from the ectoderm).

The detachment of the endoderm from the ectoderm occurred after the addition of a few drops of a mixture of alcohol and acetic acid (2 cm³ 70% alcohol + 2 cm³ 5% acetic acid + 15 cm³ distilled water). This mixture gently dissolves the mesogloea so that the endoderm could be carefully removed with the help of a wolfram needle, portion by portion, without harming the ectoderm. Afterwards

Distances (μm) of the nuclei of different ectodermal cell-types from the mesogloea measured on histological cross-sections (7 μm) of the body column of *Hydra attenuata*

Nuclei of:	*n*	Average distance (μm) from the mesogloea (standard deviation)
Epithelio-muscular cells	70	14 ± 7
Interstitial-cells	209	16 ± 9
Stenoteles:		
Nematoblasts	76	14 ± 8
Nematocytes	30	15 ± 10
Desmonemes:		
Nematoblasts	60	11 ± 7
Nematocytes	45	5 ± 4
Isorhizas:		
Nematoblasts	10	23 ± 12
Nematocytes	30	28 ± 18

[1] These investigations, forming the part of a program concerned with the regulation of nematocyte-production, were supported by the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' (No. 3.205.69).

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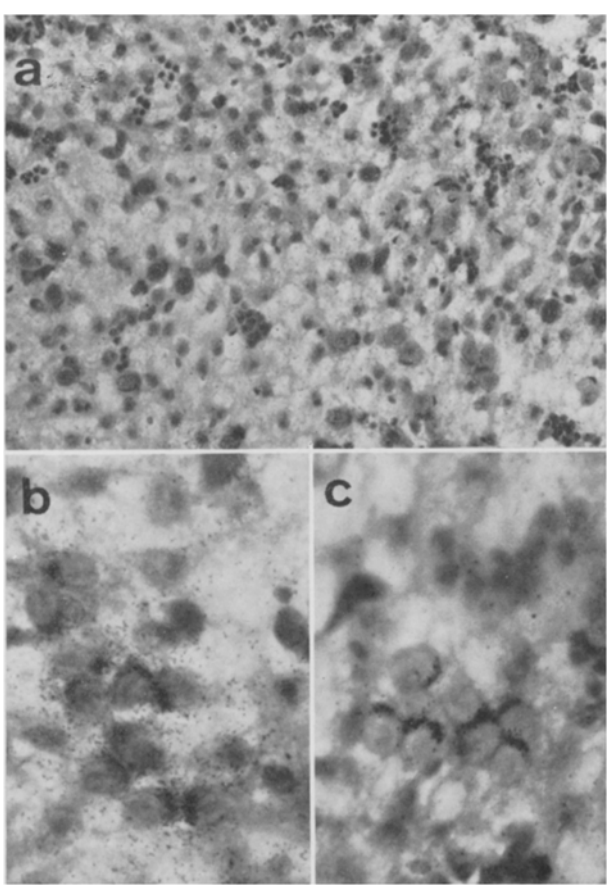
the volume of liquid covering the endoderm-free ectoderm sheet was reduced by means of filter paper. This procedure brought about a further extension of the ectodermal sheet. The specimen was then left to dry and to adhere to the surface of the albumin-coated slide. Consequently the slides could be handled and processed like mounted histological sections. The specimens were fixed with Carnoy and, if not processed further for autoradiography, stained with Mayer's hemalum, dehydrated, left to dry and covered with Malinol. Figure 1a shows a fraction of such an extended and stained whole-mount of *Hydra* ectoderm. All cell types except nerve cells can be clearly identified by the characteristic appearance of their nuclei.

For radioactive labelling of the nuclei, the living polyps were injected with 1 µl of ^{14}C-thymidine[7] solution (0.03 mC/cm^3) or with 1 µl of ^{3}H-thymidine[8] solution (0.03 mC/cm^3). The injections, which were carried out immediately after feeding of the specimens[9], were made by pushing the injection needle through the body wall. The small opening closed immediately after the withdrawal of the needle. The autoradiographic examination of such labelled animals was performed on whole-mounts of isolated and extended ectoderm prepared as described above. For autoradiography[10,11] the Carnoy-fixed ectodermal preparations were coated with stripping film (Kodak AR-10), and exposed at 4 °C for 11 (^{14}C-thymidine) or 14 (^{3}H-thymidine) days. After developing (Kodak D19b) the slides were stained with Mayer's hemalum. In order to eliminate at least part of the dye incorporated in the gelatine, the slides were immersed for 30 sec in 0.1% HCl.



Whole-mounts of isolated and extended ectoderm of *Hydra attenuata*, stained with hemalum. a) Low magnification of part of a whole-mount. ×120. b) Autoradiograph of a cluster of stenotele nematoblasts labelled with ^{14}C-thymidine. ×320. c) Autoradiograph of a cluster of stenotele nematoblasts labelled with ^{3}H-thymidine. ×320.

When using ^{3}H labelled precursors, one is confronted with the problem of adjusting the thickness of the specimen to the short range of the β-rays. Since we could not determine the thickness of our ectodermal whole-mounts, i.e. the distance between the nuclei imbedded in the tissue and the photographic emulsion, we determined these values on histological cross-sections made from the body column of *Hydra attenuata*.

Polyps fixed for histological purposes normally constrict during fixation. Consequently their ectoderm and endoderm are considerably thicker than in an extended stage. On such histological sections we measured the distances between the nuclei of various ectodermal cell types and the mesogloea. The values given in the Table show that most of the nuclei were located within a range of 40 µm from the mesogloea. Only 10 (1.9%) of the 530 nuclei examined were situated outside this range.

As the whole-mounts of the ectoderm were fixed in an extended stage and left to dry before being processed further (see above), we could assume that the total thickness of these preparations and the distance between the nuclei and the photographic emulsion were much reduced, falling within the range of the β-particles, which for ^{14}C according to ROGERS[11], is 40 µm. By using ^{14}C-thymidine all labelled nuclei will, therefore, be identified on the photographic emulsion so that this radioactive precursor can be considered as being reliable for quantitative investigations.

The ^{14}C label has, however, the disadvantage of producing widely scattered groups of silver granules (Figure 1b), which sometimes makes it difficult to decide which of two adjacent nuclei is actually labelled. In this respect ^{3}H-thymidine gives much clearer pictures (Figure 1c), but it has an even more restricted range of action (max. 7 µm, Documenta Geigy[12]). In whole-mounts, the danger of labelled nuclei, especially those deeply imbedded in the ectoderm, not showing up autoradiographically is increased. This is particularly true for specimens the thickness of which is not known, as is the case for our ectoderm whole-mounts. The ^{3}H-thymidine method can therefore be used only for qualitative purposes.

To summarise, the new method using whole-mounts of isolated and extended ectoderm of *Hydra* constitutes, with or without autoradiography, a relatively easy way of examining quantitatively and qualitatively the two-dimensional arrangement of cells within the body ectoderm under normal as well as experimental conditions. Unfortunately the endodermal sheet is not coherent enough to be isolated in a similar manner.

Zusammenfassung. Eine Methode wird beschrieben, die es erlaubt, Totalpräparate von ausgebreitetem, isoliertem Ektoderm von *Hydra* autoradiographisch zu untersuchen.

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