

Nahrung auf, und 2 derselben erreichten das Stadium, in dem das 3. und 4. Fortsatzpaar mit dem dazugehörigen Skelett angelegt war.

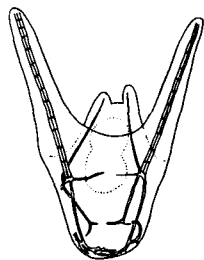


Abb. 3. 10 Tage alter Pluteus von *Sphaerechinus granularis* (Vergrößerung 150:1).

Die Skelette dieser 15 Merogone weisen rein väterlichen Typus auf (keine Gitterstäbe, keine oralen Scheitelstäbe). In einigen Fällen waren Zusatzzacken vorhanden, wie das bei *Psammechinus* und *Paracentrotus* häufig vorkommt. Die Abbildungen 1–3 zeigen das Skelett von Mutter (*Sphaerechinus*), Vater (*Paracentrotus*) und Merogon.

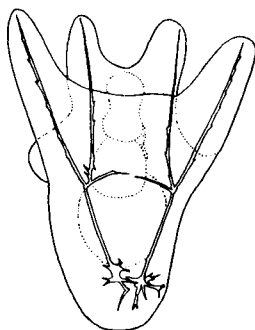


Abb. 4. 18 Tage alter Merogon von *Sphaerechinus granularis*-Plasma und *Paracentrotus lividus*-Kern (Vergrößerung 150:1).

Die relative Länge der Oral- und Analstäbe folgt dem *Psammechinus*-(*Paracentrotus*-)Typus, nicht dem *Sphaerechinus*-Typus.

Die Wimperepauletten der älteren Merogone weisen *Paracentrotustypus* auf.

Die Larvenform ist bei den noch jungen Merogonen rein väterlich, mit spitzem Scheitel. Die Scheitelstäbe weisen die typische Keulenform auf. Dann aber rundet sich noch im 4-Fortsätze-Stadium der Scheitel ab, wie es für *Sphaerechinus* charakteristisch ist (Abb. 2), es entsteht über den keulenförmigen Enden der Scheitelstäbe ein Hohlraum, in den lange Zacken der Körperstäbe hineinwachsen, wie sie normalerweise weder bei *Psammechinus* (*Paracentrotus*) noch bei *Sphaerechinus* vorkommen (Abb. 4). Die Fortsätze der älteren Merogone werden mehr breit und lappenförmig, wie es für *Sphaerechinus* charakteristisch ist.

L. VON UBISCH

Paradis, Norwegen, den 7. Mai 1953.

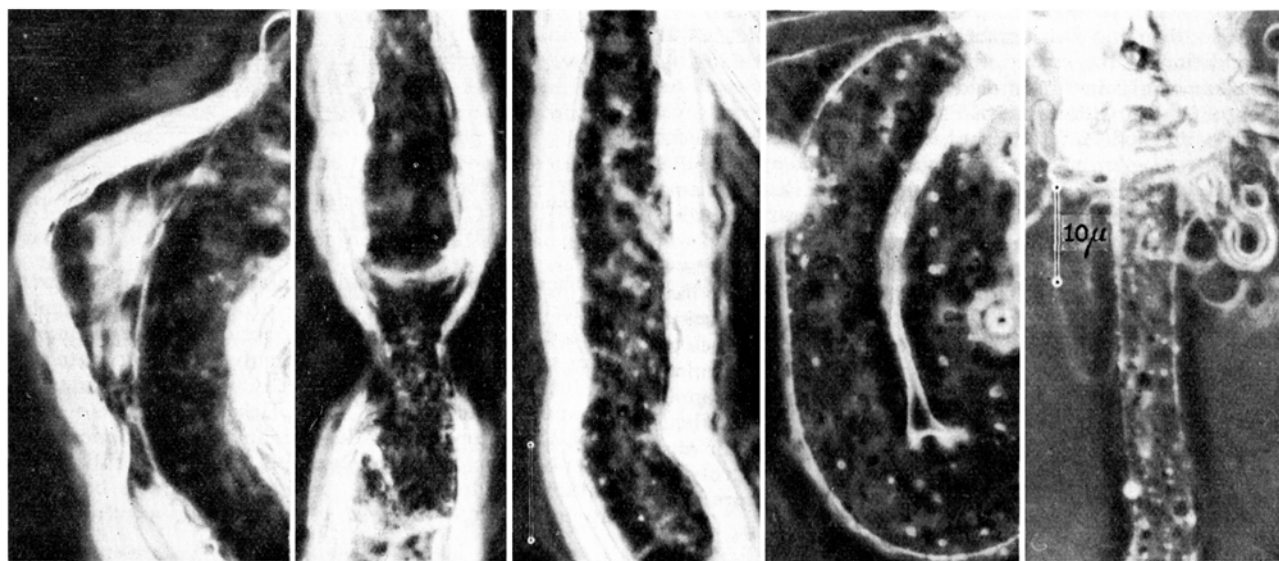
#### Summary

De-nucleated eggs of *Sphaerechinus granularis* were fertilised with sperms of *Psammechinus microtuberculatus* and *Paracentrotus lividus* (heterosperm merogones). It was possible to culture 15 embryos up to the pluteus stage. The skeletons, the relative lengths of the first pair of lobes, the body form of the young merogones and the ciliated bands of the older merogones all show paternal characters. In the older merogones, the vertex was rounded and the lobes more lobulate than is characteristic for *Sphaerechinus*.

#### Living Nerve Structure Seen by New Light Optics

With the light microscope arrangement of one of us<sup>1</sup>, some details of the fresh, normal nerve fibre can be revealed. The findings are almost identical in mammalian and amphibian nerves. Myelin sheaths frequently split into lamellar structures, especially at sharp bends and other minor traumata. Vesicular bodies ranging from 0.3 to 2  $\mu$  are scattered throughout the entire axon, in

<sup>1</sup> A. WILSKA, Nature 171, 353 (1953).



a

b

c

d

e

Fresh mouse nerve preparations showing: (a) a bend with the myelin sheath partially detached from the axon and laminating at the outer curvature, (b) a node of Ranvier, (c) intact axon structure, (d) extruded axoplasm, (e) the axon lightly pulled out from the myelin tube.

agreement with the electron-microscopic observations of FERNÁNDEZ-MORÁN<sup>1</sup>. These bodies make up some 2 to 3% of the total volume of axoplasm. Most of the bodies are more refractive than the surrounding axon structure. The axoplasm is easily extruded from the cut end of a fibre, yet its structure remains coherent. The axon can easily be pulled out from the myelin tube and stretched to several times its original length without breaking. In a stretched state the vesicles become ovoid or fusiform.

As noted by FERNÁNDEZ-MORÁN, previous light-microscopic research has failed to show any details in the normal, fresh axon, not even with the use of dark field, polarization or phase contrast.

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*Institute of Physiology, University of Helsinki, March 15, 1953.*

#### Zusammenfassung

Durch Anwendung eines abgeänderten Lichtmikroskops werden Strukturen in lebendfrischen Nervenfasern, die lichtoptisch bisher unsichtbar gewesen sind, deutlich gesehen.

<sup>1</sup> H. FERNÁNDEZ-MORÁN, *Exp. Cell Res.* **1**, 143, 309 (1950); **3**, 5 (1952).

### Separation and Identification of the Degradation Products of Purines and Nucleic Acids on Acid Hydrolysis by Circular Paper Chromatography

In the course of our investigations on the separation and identification of purine and pyrimidine bases by circular paper chromatography<sup>1</sup>, we investigated the nature of the products formed after acid hydrolysis of purines and nucleic acids, using the reagents, sodium 1:2 Naphthoquinone 4-sulphonate (FOLIN's reagent) in acetone<sup>2</sup>, ninhydrin in acetone<sup>1</sup> and the contact printing technique using photographic paper and filtered ultraviolet light<sup>3</sup> for the identification of the substances separated on the chromatogram. While this work was in progress, FRICK<sup>4</sup> reported the formation of amino acids in acid hydrolysates of adenine by paper chromatography. He also observed the formation of two ultraviolet spots in addition to the strong ninhydrin-positive spot in the position of glycine after acid hydrolysis of adenine. In view of the above findings of FRICK and our observations on the marked differences observed in the hydrolysis products of adenine, nucleic acids, adenosine, adenylic acid, adenosine-3-phosphoric acid on the one hand and those of the other purines and pyrimidines on the other prompted us to report our observations.

Adenine (25 mg) was hydrolysed with (3 cm<sup>3</sup>) 6 Normal hydrochloric acid by autoclaving at 15 lbs. pressure for 6 h. After removing the acid, the solution was spotted in 3 aliquots of 5  $\mu$ l on the circumference of a circle drawn at the centre of the filter paper (Whatman No. 1, 24 cm diameter) and developed with n-butanolic-acetic-acid-water according to the procedure described by GIRI and RAO<sup>1</sup>. After drying, the chromatogram was cut into three sectors, each containing the hydrolysis products of adenine separated. One of the sectors was

sprayed with 0.2% ninhydrin in acetone<sup>1</sup> and the other with FOLIN's reagent<sup>2</sup>. The third sector was placed on a photographic paper and exposed to filtered ultraviolet light and developed according to the procedure described by MARKHAM and SMITH<sup>3</sup> (Fig. 1).

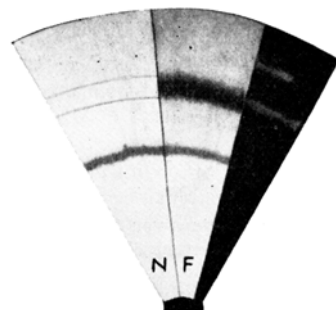


Fig. 1.—Different sectors of circular paper chromatogram of adenine hydrolysate treated as indicated below. *N* Sprayed with ninhydrin. *F* Sprayed with FOLIN's reagent. *U* Contact print of the sector taken in the ultraviolet.

It was observed that the adenine hydrolysate gives three clear bands on the chromatogram: (1) The band with the lowest  $R_f$  value (0.38) gives purple colour with ninhydrin and green colour with FOLIN's reagent and occupies the position of glycine on the chromatogram; (2) the second band with  $R_f$  value (0.69) higher than the first band gives faint yellow colour with ninhydrin (outlined with pencil in Fig. 1) and intense purple with FOLIN's reagent. It is also characterised by ultraviolet absorption as it appears on the contact print; and (3) the third band which appears on the sector obtained by contact printing technique is due to unhydrolysed adenine. Figure 2 is the reproduction of the chromatogram of adenine and adenine hydrolysate obtained by contact printing technique. It shows the formation of another band characterised by the ultraviolet light absorption, in addition to the one relating to adenine.



Fig. 2.—A contact print of a sector of a circular paper chromatogram of adenine and adenine hydrolysates taken in the ultraviolet using a chlorine filter. *A* Adenine, *A(h)* Adenine hydrolysate.

Other compounds of adenine and nucleic acids were similarly subjected to acid hydrolysis and chromatographed. Figure 3 is the reproduction of the chromatogram showing the bands of the substances separated on the filter paper, present in the hydrolysates of adenine, ribonucleic acid (R.N.A.), Desoxyribonucleic acid (D.N.A.), Adenosine, Adenylic acid, and Adenosine-3-Phosphoric acid. It was observed that all the substances containing adenine give, in addition to the band occupying the position of glycine band, another band

<sup>1</sup> K. V. GIRI and N. A. N. RAO, *Nature* **169**, 923 (1952); *J. Ind. Inst. Sci.* **34**, 95 (1952).

<sup>2</sup> K. V. GIRI and A. NAGABHUSHANAM, *Naturwissenschaften* **23**, 548 (1952).

<sup>3</sup> R. MARKHAM and J. D. SMITH, *Biochemical J.* **45**, 294 (1949).

<sup>4</sup> G. FRICK, *Nature* **169**, 759 (1952).

<sup>1</sup> K. V. GIRI and N. A. N. RAO, *Nature* **169**, 923 (1952); *J. Ind. Inst. Sci.* **34**, 95 (1952).

<sup>2</sup> K. V. GIRI and A. NAGABHUSHANAM, *Naturwissenschaften* **23**, 548 (1952).

<sup>3</sup> R. MARKHAM and J. D. SMITH, *loc. cit.*