ORIGIN OF THE EOLIDIAN NEMATOCYSTS FROM THE STANDPOINT OF REGENERATION

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FOUR FIGURES

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The question as to the origin of the nematocysts of the molluscan eolids was first raised in the middle of last century. T. S. Wright (1858) was the first to maintain, from his comparative observation of the nematocysts and the hydroids eaten by the animals, that they are exogenous. Unfortunately this important suggestion was overlooked until the appearance of Grosvenor's paper in 1904, and during this interval R. Bergh (1862) and others maintained the endogenous theory of their origin. G. H. Grosvenor (1904) examined nematocysts precisely by the improved histological methods of his day and at the same time conducted experiments in feeding the animals with hydroids, and gave an excellent account of the origin of the nematocysts, which he claimed was exogenous. L. Cuénot (1907) and O. C. Glaser (1910) concurred in this statement of Grosvenor as to their origin. However, more recently A. Labbé (1923) brought forward a theory which reconciles these two extreme views, stating that the nematocysts of the smaller and presumably younger cerata are endogenous, while those of the larger and older cerata are exogenous. A. Naville (1926) opposed this view and reverted to the exogenous origin of the nematocysts in both larger and smaller cerata.

Experimental researches have also been attempted, the relationship existing between the nematocysts in the cnidosac and those found in the diet being particularly studied. Cuénot (1907) tried to settle this problem by his cerata regeneration experiment, cutting off the tip including the cnidosac. He hoped to obtain in this way cerata free

from nematocysts, but the result was the production of cerata only partly free from nematocysts. The procedure adopted in this experiment seems, however, to be imperfect in several points; in particular, the origin of the nematocysts which are said to be still partly formed is somewhat doubtful. Glaser (1910) suggested that the uncertainty of Cuénot's result could be eliminated by starving the experimental animal for several days before operation. The present study was undertaken to verify this statement of Glaser by repeating Cuénot's regeneration experiment. The experiment was carried at the suggestion of Prof. Dr. Yô K. Okada at the Misaki Marine Biological Station during my short stay there last summer.

OBSERVATION

Amphorina sp.; This eolid feeds on *Tubularia mesembryanthemum*. In addition to a pair of labial tentacles and a pair of rhinophores, the animal possesses 7 oblique rows of cerata symmetrically placed on either side of the body, each being composed of 3-5 papillæ. Behind the rhinophores and on the side of the body, the cerata are small, but in the middle region and especially on the median line of the back they are large. Each ceras ends in a cnidosac containing nematocysts absolutely identical to those found in *Tubularia*. In the experiments,

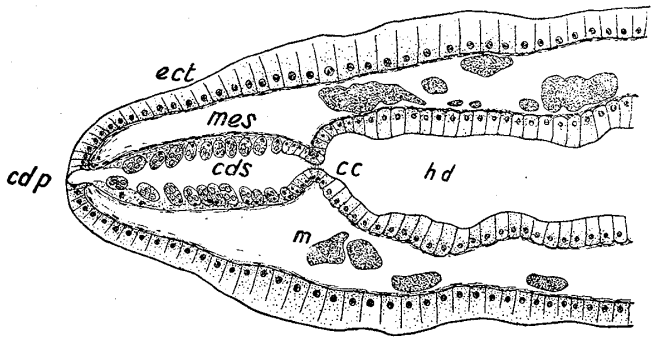

Fig. 1—Median longitudinal section of a ceras (*Amphorina* sp.), showing the internal structure of the cnidosac. × 100. *c c* ciliated canal; *cd ct* cnidocyst; *cd p* cnidophorous pore; *cd s* cnidosac; *c m* cellular mass; *ect* ectoderm; *h d* hepatic diverticulum; *m* muscle; *mes* mesenchyma; *nph* nematophagous cell; *r cds* regenerated cnidosac; *w* wounded place in ectoderm.

I selected only larger specimens from 8 to 10 mm. long, and starved them for about 5 days before operation. Thus I could entirely eliminate the nematocysts from the digestive tract of the experiment animals.

In the first series of experiments I cut off with a pair of sharp scissors the distal end of the cerata including the cnidosac. They would close almost instantly. In spite of the autotomous character of this appendage in general, I could manage to prevent it from being broken off from the proximal part. The cerata gradually grew again and about 10 days after the operation they were sufficiently large and produced cnidosacs at the distal ends. The new cnidosacs were white and comparatively transparent, so that the ingested nematocysts could be easily recognized even from without. Five days later i.e. on the 15th day after operation, the cerata had nearly attained the original size and restored their normal shape and structure. If, however, the experiment animals were not fed with hydroids they could scarcely survive 5 days, and generally died before the end of that time. It was impossible, therefore, to obtain perfect regeneration of the cerata free from nematocysts in this species.

After operation, the ectoderm contracts exceedingly around the wound and closes it completely as is usual in such cases. The cut end of the hepatic

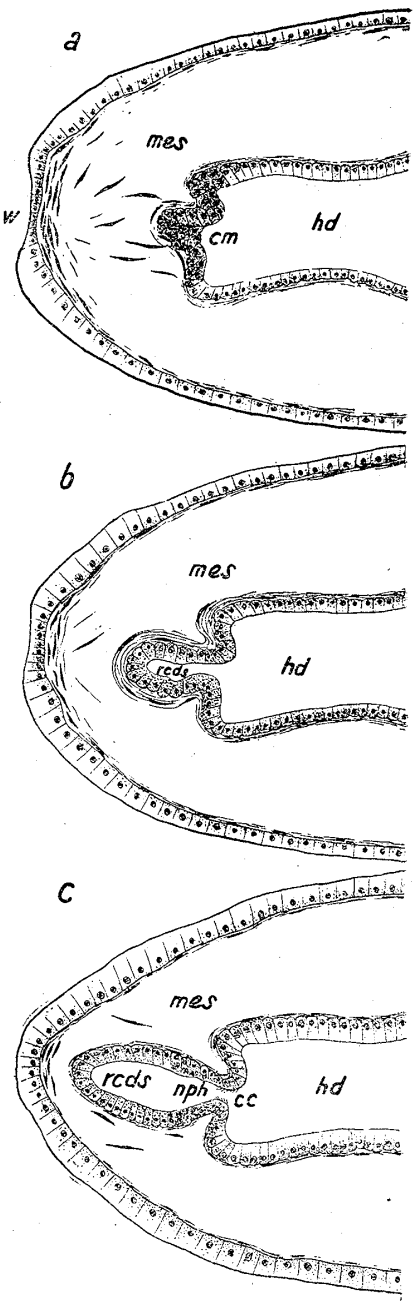

Fig. 2—Regeneration of a ceras in longitudinal sections, showing the condition of (a) about 3 days, (b) 7 days, (c) 10 days after operation. × 100.

diverticulum also closes its wound independently. Thus there is a double closing on the operated surface. Soon after this formation the ceras increases in its length, the growth being accompanied by a cellular proliferation from the distal part of the hepatic diverticulum (text-fig. 2 a). Since the newly formed cells stain particularly well with Heidenhain's hematoxylin, the regenerated part is easily distinguishable. At first the new cells gather together in a mass and layers of enlarged mesenchyme cells clothe it all around, but before long there appears a space in it and a hollow structure is produced (text-fig. 2 b). This represents the cnidosac in a regenerated ceras. The condition may be well seen in text-figure 2 b which is drawn from a section of a 7-day specimen. About 10 days after operation, cilia make their appearance in the narrow passage at the base of the new cnidosac, and the cells located in the proximal part of the new cnidosac begin to ingest the tubularian nematocysts which have been carried in from the hepatic diverticulum through the ciliated canal (text-fig. 2 c). By this time the layers of mesenchyme cells around the regenerating part of the hepatic diverticulum have become thinner and are reduced almost to the original condition of the connective tissue. The cells that have taken up a number of nematocysts grow gradually and give rise to the cnidocysts. Thus on the 15th day from beginning of the experiment, the regenerated cerata of the eolid had completely regained their full size and bore nematocysts in their cnidosacs.

Those eolids which were not fed with *Tubularia* after operation followed the same processes of regeneration. The closing of the wound and cellular augumentation from the hepatic diverticulum take place quite normally, but these regenerative phenomena proceed more slowly than in the preceding case; the internal condition of a 5-day specimen scarcely reaches that of 3-day material of the other case.

In the second series of experiments I cut off the entire cerata from their proximal part and followed the process of regeneration as before. In this case, complete regeneration of the cerata was also obtained. Only it took, as might be expected, a much longer time, viz. about 17-20 days, for the ceras to become a perfect organ with the cnidosac containing the nematocysts. Those control animals which had not been fed died out before 5th day, as in the previous experiment.

Aeolidiella takanosimaensis: Besides the labial tentacles and rhinophores on the head, this eolid possesses 13 oblique rows of 3-5 cerata on each side of the body. It was found among gravel in shallow water near the Station depositing eggs, so that its feeding habit was not clear. However, judging from the shape of the nematocysts in the cnidosac, it may feed upon some sea-anemones. In spite of the small number

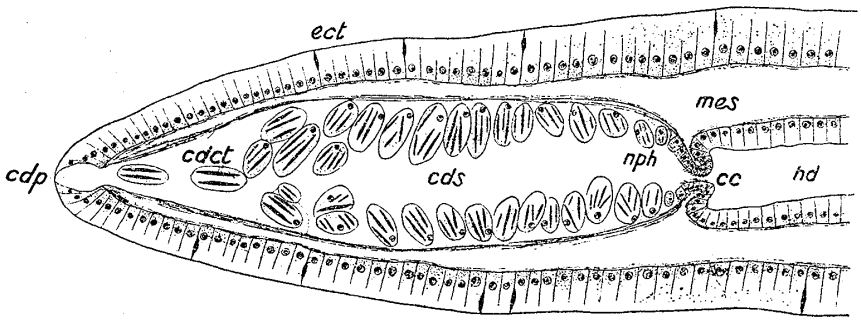

Fig. 3—Longitudinal section of a ceras (*Aeolidiella takanosimaensis*), showing the internal structure of the cnidosac. × 100.

of specimens collected, they seemed, from their considerably larger size (15-17 mm.) very promising material for a repetition of the experiment of regeneration of the cerata in starved animals, the experiment which had failed in the preceding case. The experiment animals were starved for 5 days before operation and the distal part of the cerata was cut off, as before. This time the process of regeneration took place quite well, and about 10 days later the cerata regained their original

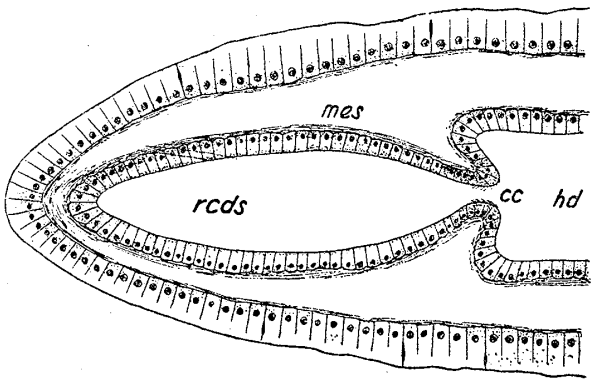

Fig. 4—Longitudinal section of a regenerating ceras under starvation for about 10 days after operation. × 100.

length. Moreover each ceras produced a well formed cnidosac at its distal end (text-fig. 4). However, no single nematocyst was found to be contained in the sac though otherwise the structure was entirely the same as in the normal animal (compaire with text-fig. 3).

REMARKS

In the case of *Amphorina* sp. used in the present experiments, it is well proved that the nematocysts of eolids are entirely identical to those of the coelenterates eaten by the animal, as has been shown by Wright, Grosvenor, Cuénot, Glaser, Labbé and Naville in the European and the American species. Some of these authors have followed direct transference of the nematocysts from the body of coelenterates or have even succeeded in changing the kind of nematocysts in the cnidosac by altering the diet. That those eolids, generally feeding upon eggs and larvæ of fish, are *à priori* devoid of nematocysts is also good evidence for the exogenous origin of the nematocysts. The view that their origin is exogenous is strongly corroborated by the present experiments on cerata regeneration. Regeneration of the latter in the presence of coelenterate diet is always accompanied by a normal formation of the cnidosac with nematocysts, but in starvation or in the absence of the coelenterate diet, the nematocysts never appear in the regenerated cnidosac, although this organ may be normally produced as it is in *Aeolidiella takanosimaensis*. A similar experiment has already been attempted by Cuénot, who obtained cerata only partly devoid of nematocysts. His failure to produce cerata entirely free from nematocysts must be due to the presence of capsules remaining in some parts of the digestive tract, since Cuénot did not starve the experimental animals before operation.

Labbé's compromise theory, as mentioned above, claims that the nematocysts have a dual origin, and this was immediately opposed by Naville on the evidence of his careful histological examination. However, both these authors hold the view that the smaller cerata behind the rhinophores or on each side of the body are embryonic, while the larger ones found in the middle part of the back are adult, and that the cerata in the embryonic state are carried upwards and grow up to

the adult state in the median line of the back. Naville laid particular stress upon the structural differences between these two kinds of cerata.

So far as my observation is concerned, the smaller cerata regenerate as well as the larger ones and in both cases the nematocysts are always derived from coelenterates eaten by the animals. When all the larger cerata are taken off, the smaller ones remaining on the side of the body actually grow large and reach nearly the same size as the large cerata. Thus animals having only large cerata can be derived. During this experiment however, I could not observe such a phenomenon as the gradual carrying upwards of small cerata towards the median line of the back and the growth of new cerata on each side of the body. There are, no doubt, some structural differences between the larger and the smaller cerata, but I think this may rather be due to specialization of their function according to the place where they are situated.

SUMMARY

Amphorina sp. and *Aeolidiella takanosimaensis* were used for the present experiments. The nematocysts found in these species are entirely identical to those of the coelenterates they eat.

The cerata can regenerate on either fed or starved animals. In the first case the regenerated cnidosac always contains nematocysts, but in the second case it is devoid of them. Thus the exogenous origin of the eolidian nematocysts is firmly supported from the experimental side.

LITERATURE CONSULTED

Cuénot, L. 1907 L'origine des nématocystes des Eolidiens. Arch. Zool. exper. et génér. (4 me serie), tom. 6, p. 73.

Glaser, O. C. 1910 The nematocysts of Eolids. Jour. exper. Zoöl. vol., 9, p. 117.

Grosvenor, G. H. 1904 On the nematocysts of Aeolids. Proc. R. Soc. London, vol. 72, p. 462.

Labbé, A. 1923 La genèse des nématocystes des Nudibranches. C. R. Acad. Sc. Paris, tom. 176, p. 1508.

Naville, A. 1925 Sur la présence d'une Embletonia dans le Canal de Caen à la mer; origine exogène de ses nématocystes. C. R. Acad. Sc. Paris, tom. 181, p. 812.

—— 1926 Notes sur les Eolidiens. Un Eolidiens d'eau saumâtre. Origine des nématocystes. Zooxanthelles et homochromie. Rev. Suisse Zool., tom. 33, p. 251.