Communication présentée le 28 mai 1960.

THE NEMATOCYST OF HYDRA (Part II).

THE MATURATION OF NEMATOCYSTS IN HYDRA

by

Allison L. BURNETT,

Laboratoire de Zoologie et de Biologie animale,
Université Libre de Bruxelles

ACKNOWLEDGEMENT.

This work was sponsored by a post-doctoral grant awarded to the author by the National Science Foundation of the United States of America. This grant allowed the author to work in the laboratory of Dr. Paul Brien for a period of one year.

The author wishes to express his deepest thanks to Dr. Paul Brien whose many suggestions and kind encouragement were a great source of stimulation to the author during the progress of these studies.

INTRODUCTION.

Most papers in scientific literature which deal with the subject of the development of the nematocysts in coelenterates will contain somewhere in their pages the terms « Mature » and « Immature » nematocysts. However, no single author has sharply defined what is meant by a mature nematocyst or has given a definite criterion which will differentiate between immature and mature nematocysts. In recent years, several excellent studies on the nematocyst have been conducted with the aid of an electron microscope. Outstanding among these studies are those of SEMAL-VAN GANSEN (1954), BOUILLON et al. (1958).

Slautterback and Fawcett (1959), and Chapman and Tilney (1959 a, 1959 b). Although, these authors have presented in much detail a description of the fine structure and development of the nematocyst capsule, thread and operculum, the process of nematocyst maturation remains incomplete. For it must be held in mind that the maturation process of the nematocyst is not merely one of morphological development, but one of chemical development as well. Clearly, it is possible to have a structurally perfect nematocyst which is still in the process of elaborating the poison within the capsule. Therefore, a thorough study of nematocyst maturation must include joint chemical and anatomical studies.

A simple definition of a mature nematocyst might be as follows, « Any nematocyst which is capable of discharging is mature ». However, Weill (1934) and Chapman and Tilney (1959 b) have stated that immature nematocysts are capable of evaginating. It is regretful that the latter authors were not more specific in describing the stage when a nematocyst is first capable of discharging, but it is obvious that these investigators have indeed observed discharged nematocysts which were not fully sculptured.

Where does one expect to find a mature nematocyst in coelenterates? It appears most likely that mature nematocysts will be found in the tentacles, the place where they will be employed to the greatest advantage rather than in the gastric region of the animal. A simple longitudinal section through a hydra will reveal, as Kepner (1943) has stated, that nematocysts are found in the process of formation in the distal or oral ¾ of the body. Semal-Van Gansen (1951) has presented a much more elaborate scheme for general nematocyst distribution. She considers each region of the body of hydra separately and offers a general account of the number and kinds of nematocysts found in each region. She states that the epidermis of the gastric column is rich in cnidoblast cells while the regions of the base and peduncle are sparsely populated by or deprived of these cells. In the tentacles, where the nematocyst batteries are formed, this author considers the nematocysts to be « ripe ». Once a nematocyst is part of the nematocyst battery, the cell containing the nematocyst is no longer called a cnidoblast by Semal-Van Gansen but a nematocyte. The nematocyte always has its position in a nematocyst battery located in the tentacles of the hydra; the French equivalent for the term nematocyst

battery is « bouton urticant ». Thus, we may assume from SEMAL-VAN GANSEN's description that a nematocyst in the gastric region is in the state of differentiation whereas a nematocyst in a tentacle battery is completely formed.

However, if one makes a longitudinal section through a hydra and compares a stenotele in the tentacle with a stenotele in the gastric region, it will be found, at least as far as can be determined with the light microscope, that both contain a fully elaborated thread, capsule, and operculum. From this observation an important question arises; is there a difference between a nematocyst in the gastric column where the nematocyst has been formed and a nematocyst in the tentacle battery where it will be employed ?

This paper purports to answer this question on the basis of histochemical tests applied to whole animals. By examining whole animals it is possible to observe the general distribution and chemical pattern of all four of the types of nematocysts found in hydra intact.

MATERIALS AND METHODS.

The animals employed in these studies were healthy, adult specimens of the brown hydra, *Pelmatohydra oligactis*. Methods for rearing these animals have been described in a previous paper (See BURNETT, 1959).

The animals which were chosen for histological examination were prepared in the following manner :

1. Relax the hydra for one minute in a solution of 10 % alcohol.
2. Fix for ½ hour in 100 % alcohol.
3. Bring the hydra down through a graded series of alcohols to water and immerse them for one or two minutes in a methylene blue solution (concentration .01 %). According to the type of nematocyst desired to stain, it is necessary to stain the hydra in solutions of different p.H's. The method adopted for the present study has been that of SINGER and DEMPSEY (1947— taken from PEARSE, 1954, p. 434).

   Methylene blue solutions were buffered in the present study with Micrhome Buffer Tablets purchased from Edmund Gurr Ltd., Upper Richmond Road W., London, S.W. 14.

England. Buffer solutions of the following p.H's were employed in the following study: 3.6, 4, 5, 7, 8, 9, 10.

4. Differentiate in 30 % alcohol until the bodies of the hydra are stained a light blue. At this time the nematocysts should be staining densely.
5. Bring rapidly to 100 %alcohol to prevent further loss of stain.
6. Clear in xylene or toluene.
7. Mount whole animals in balsam.

This method provides for a rapid staining procedure which may be considered specific for nematocysts since these structures concentrate the stain to a much greater extent than any other structure in hydra. Slides prepared by this method will often begin to fade after a period of from one to two months. therefore, it is recommended that the slides be examined within a week or two after preparation.

It was also necessary during these studies to graft the tissues of one hydra to the tissues to another one or two days before the animals were fixed for histological study. The graft in all cases consisted of the peduncle of one animal which was placed between the growth region and budding region of a normal animal. For a description of these grafting techniques see Burnett (1959).

Observations.

A group of 250 hydra were stained in methylene blue solutions of various p.H's and then observed under a light microscope. At p.H 8, the stenoteles which were located in the peduncle of the animal plus the stenoteles in the distal ¾ of the tentacles were stained intensely. Stenoteles located at the bases of the tentacle plus those in the gastric and budding regions of the animal, however, were unstained at this p.H. The staining of isorhizas (*) followed a similar pattern. At this p.H desmonemes had only a slight affinity for the stain: those which did impregnate the stain were in the distal ¾ of the tentacles.

At p.H 9 the stenoteles and isorhizas stained in the same manner as those at p.H 8 except that the nematocysts in the

(*) Examination of whole mounts of *P. oligactis* stained with methylene blue fails to reveal any criterion whereby it is possible to distinguish a holotrichous isorhiza from an atrichous form, thus, the general term « isorhiza » will be employed in this paper.

proximal $^1/_3$ of the tentacles were unstained on this occasion, and nematocysts stained only in the lower $^2/_3$ region of the peduncle. There was no staining of desmonemes at this p.H.

At p.H 10 only the stenoteles had an affinity for the stain, and these stenoteles were located in the extremities of the tentacle (Fig. 3 and 4). There was no staining of stenoteles which were located in the peduncle.

Staining at p.H 3.6. presented quite a different picture. At this p.H only the isorhizas stain. They stained intensely in the body region as well as the tentacles. Stenoteles were generally unaffected at this p.H (Fig. 1), however, there was, on occasion, a light staining of the stenoteles in the basal regions of the tentacles and in the gastric region.

At p.H 4 isorhizas continued to stain intensely in all areas of the body. Desmonemes in the tentacles also stained at this p.H. There is an increasing affinity for the stain by stenoteles in the basal regions of the tentacles and in the gastric region.

At p.H 5 there was a fairly intense staining of stenoteles in the gastric region of the animal and also in the lower ¼ of the tentacles. Desmonemes and isorhizas continue to stain throughout the tentacles and the body region. Also there was a dense staining of the isorhizas in the peduncle. Desmonemes are lacking in this region.

At p.H 7 there is a general staining of stenoteles in the entire body of the animal, however, those stenoteles in the tentacles concentrated the stain to a greater extent than similar structures elsewhere in the body (See Fig. 4). Holotrichous isorhizas in the body region either do not stain or stain very feebly. Those in the tentacles stain intensely. Also it was noticed that isorhizas in the developing tentacles of the bud stained whereas those in the gastric region of the bud did not.

A group of animals which had been starved for 14 days were stained at p.H 8, and there was no deviation in the staining pattern from the normal animal. Stenoteles in the lower ¼ region of the stenoteles still failed to concentrate the stain.

The peduncles from 5 hydra were grafted between the growth regions of the budding regions of 5 normal animals. Two animals were stained at p.H 8 on the first day after the graft and 3 animals were stained on the second day. In all cases the only stenoteles in the gastric region which had an affinity for the stain were those in the regions just bordering on the grafted peduncle.

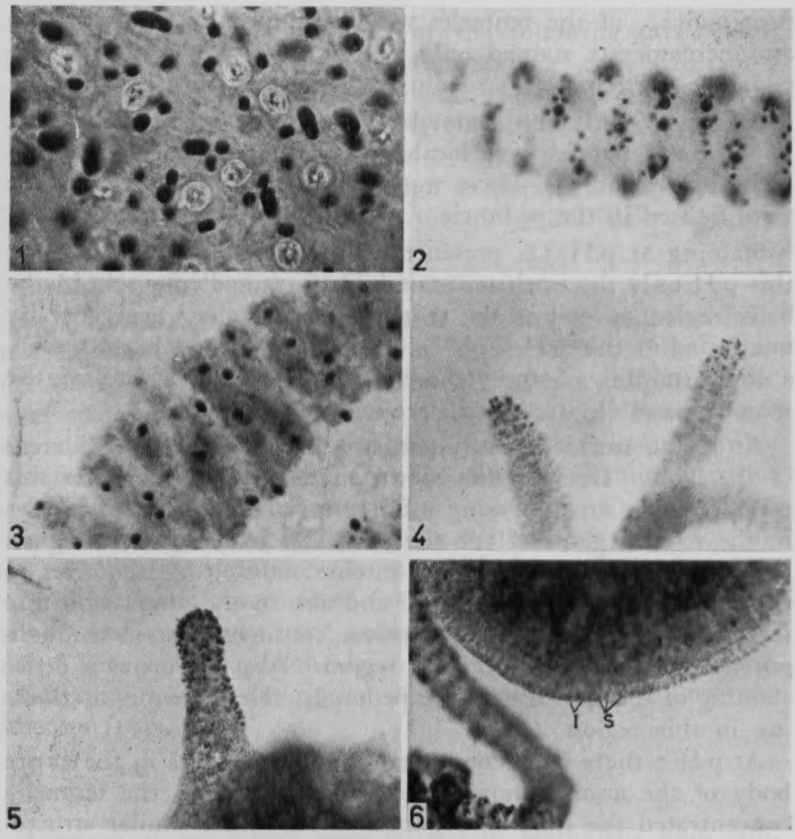

FIG. 1.

Photograph of whole tentacle stained with methylene blue at pH 3.6. Note the intense staining of the oblong isorhizas and round desmonemes and the lack of stain in the large stenoteles.

FIG. 2.

Photograph of whole tentacle stained with methylene blue at pH 7. Note the general staining of all types of nematocysts and the densely staining stenoteles in the center of each nematocyst battery.

FIG. 3.

Photograph of the extremity of a whole tentacle stained with methylene blue at pH 10. Note that only the stenoteles stain at this pH.

FIG. 4.

Photograph of the tentacles of a bud stained with methylene blue at pH 10. Note that only the stenoteles in the tip of the tentacles concentrate the stain

FIG. 5.

Regenerated tentacle of an animal which had been severed thirty hours previously through the gastric region. Note the staining of stenoteles after treatment with methylene blue buffered to pH 8.

FIG. 6

Proximal portion of a hydra which had been severed thirty hours previously through the gastric region. Note the staining of isorhizas (I) and stenoteles (S) after treatment with methylene blue buffered at pH 8.

Several hydra were excised midway between the budding region and growth region, and the resulting sections were allowed to regenerate for approximately 30 hours. The stenoteles in the buds of the developing tentacles of the proximal excised portions stained at p.H 8. The stenoteles in the developing portions of the excised distal sections stained in a similar manner at the same p.H (See Fig. 5). Isorhizas possessed a similar staining capacity as the stenoteles in both proximal and distal sections (See Fig. 6).

TEXT FIGURE I.

		pH 3.6	*pH* 4	*pH* 5	*pH* 7	*pH* 8	*pH* 9	*pH* 10
Peduncle	S	—	—	--	+	+	+ —	—
entire	D	—	—	—	—	—	—	—
	I	+	+	+	+	+	+ —	—
Growth, Gastric	S	— ?	+	+	+	—	—	—
Budding	D	—	—	+	+	—	—	—
Region	I	+	+	+	?	—	—	—
Proximal 1/4	S	— ?	+	+	+	—	—	—
Region of	D	—	+	+	+	—	—	—
Tentacles	I	+	+	+	+	—	—	—
Distal 3/4	S	—	—	—	+	+	+ —	— +
Region of	D	—	+	+	+	+	—	—
Tentacles	I	+	+	+	+	+	+ —	—
Proximal 3/4 Reg. of Tent.	S	—	— +	+ —	+	+ —	+ —	—
Distal 1/4 Reg. of Tent.	3	—	—	—	+	+	+	+
Distal 1/3 Reg. of Ped.	S	—	—	—	+	+	—	—
Proximal 2/3 Reg. of Ped.	S	—	—	—	+	+	+	—

Several whole animals were also stained in a toluidine blue solution buffered at p.H 7.5. The stenoteles in the distal ¾ of the tentacles and those in the peduncle stained a dense violet. However, the stenoteles in the gastric region stained a light pink, those in the most basal regions of the tentacles a darker pink, while those in the mid-region, i.e., between the basal region and distal ¾ of the tentacle, stained deep red. Hence, there was a gradual increase in metachromasia extending from

the base of the tentacles towards the distal ¾ region of the tentacles. Similarly, a gradient of intensity of metachromasia was witnessed between the budding region and the upper $^1/_3$ region of the peduncle with the metachromasia increasing proximally from the budding region. Text Figure I is an attempt to illustrate graphically the results of all of the foregoing staining experiments conducted at different p.H's.

It must also be mentioned that in all of the foregoing staining reactions, the stain was limited to the contents of the nematocyst capsule and had no effect upon the capsule proper. Discharged nematocysts either did not stain, or if the poison was not completely extruded from the capsule upon discharge, stained only partially.

Discussion.

It is clear from the preceeding observations that nematocysts which are located in the body or gastric region of the hydra do not possess the same properties as nematocysts located in the tentacles. Although the nematocysts in the gastric region have elaborated a capsule and stinging thread, it appears that the poison in the capsule is not fully elaborated until the nematocysts have reached the tentacles. Evidence for this is demonstrated by the fact that there is a gradual increase in acidity of stenoteles extending from the body region towards the extremities of the tentacles. A similar increase in metachromasia in these structures lends further support to this conviction.

Semal-Van Gansen (1951), elaborating a theory of Brien (1949), demonstrated that the nematocysts of hydra reach the tentacles from the body region where they have been formed, not through an active migration distally via the gastrovascular cavity as had been supposed by earlier authors, but through the normal growth processes of the animal. According to Van Gansen, actively multiplying cells in the growth region of hydra force cnidoblast cells in this region basally towards the peduncle and distally towards the tentacles. Thus, the migration is an epidermal one and also is a gradual process. Van Gansen's theory will be discussed in more detail in the following paper in this series, but for the present it may be mentioned that the fact that nematocysts show a gradual increase in metachromasia and acidity as they extend from the growth region to the tentacle region may be taken as evidence in support of this theory. If nematocysts migrated to the tentacles via the gastrovascular

cavity the process would be a rapid one, and would not expect to find a regular chemical change along the entire length of the tentacle.

If it is assumed that growth processes are responsible for the migration of nematocysts in hydra, the following question arises. Do nematocysts gradually ripen chemically as they move proximally and distally from the growth region because a fixed time is necessary for the ripening process to complete itself, or do nematocysts change chemically because of influences of the particular body region in which the nematocyst happens to be located? If a nematocyst requires a fixed time for its chemical development, then it should be possible by inhibiting growth processes to bring about the complete maturation of all the nematocysts in the body of hydra. BURNETT (1959) has shown that starvation will bring about the inhibition of growth processes since energy for cell multiplication is lacking after a period of approximately five days. In the present studies it has been shown that a hydra which has been starved for fourteen days stains similarly at p.H 8 to a normal animal. Thus, it appears that a nematocyst will complete its chemical development only when it is under the influence of the tentacle or peduncle region, or, conversely, not under the influence of the gastrict or growth region.

Support of the foregoing conviction is gained from the experiment which involved the transfering of a peduncle between the growth and budding regions of normal animals. Nematocysts bordering on the grafted peduncle rapidly increase in acidity. Furthermore, if a cut is made through the middle of a hydra, nematocysts on either side of the incision become acid within thirty hours indicating that the formation of a new peduncle or tentacles necessarily involves the « ripening » of nematocysts. A nematocyst located in the mid-region of a hydra would normally take at least a week to reach the region of the peduncle through growth processes alone.

Therefore, it is safe to assume that a nematocyst in the growth region or gastric region of a hydra, although it has elaborated its capsule, is held in a kind of chemical abeyance. Only when a nematocyst is removed from these areas or brought under the influence of the tentacle or peduncle region is this inhibition released.

The nature of this inhibition is unknown at the present time. It is a well known fact that the hypostome region of hydra

suppresses the formation of another hypostome in its immediate vicinity. It is also quite possible that there is a substance in the growth or gastric regions of hydra which suppresses the full elaboration of a nematocyst poison. As the nematocyst is pushed by growth processes to the tentacles and peduncle, this inhibition becomes less apparent. On the other hand, the tentacles and peduncle of the hydra might furnish a substance needed for the complete development of the nematocyst poison, a substance which is lacking in the growth and gastric regions.

It must also be mentioned that the nematocyst is becoming more and more acid along the entire length of the tentacle. It will be remembered that at p.H 10, only the stenoteles at the tentacle tips have an affinity for methylene blue. It is quite possible that increased acidity is a reflection of the production of a more potent poison in the nematocyst capsule. Since the nematocysts most oftened employed in the process of subduing prey are those in the extremities of the tentacles, it is of great advantage to the animal if these nematocysts are the most potent ones in the body.

On the other hand, chemical changes are also witnessed in nematocysts such as desmonemes which do not liberate any poison upon discharge. Nevertheless, the desmoneme is not a hollow structure; it contains a fluid, that nature of which is unknown at the present time. Also, the desmoneme is a highly specialized type of nematocyst. It is quite possible that the desmoneme evolved from a nematocyst that was once a penetrant, i.e., pierced the prey's tissues and liberated poison upon discharge, and although it has become greatly modified, still retains a poison inside its capsule.

It will be noticed that isorhizas stain at p.H 3.6. and also at p.H 8 in the area of the tentacles and peduncle. The conflicting nature of these results probably is due to the fact that it was impossible in these studies to differentiate between an atrichous and a holotrichous isorhiza. These two types of nematocysts may have quite different contents inside their capsules. However, Semal-Van Gansen (1951) points out that there are no atrichous isorhizas in the peduncle of the hydra. If this is true, then it is difficult to explain the staining characteristics of holotrichous isorhizas in the peduncle at highly acid and alkaline p.H's.

In conclusion, it may be safely stated that the stenotele of hydra is not capable of completely elaborating its poison until

it reaches the region of the tentacles or peduncle. The fact that the ripening process is a gradual one extending from the base to the extremities of the tentacles indicates that nematocysts reach the tentacles from the lower body regions through the normal growth processes of the animal and not by an active migration via the gastrovascular cavity. Thus, a stenotele may not be considered mature until it is located in the distal ¾ region of the tentacles.

Summary and Conclusion.

Although nematocysts in the growth region of hydra may be fully developed anatomically, they may be still considered immature since the poison contained within their capsules is not fully elaborated until the nematocysts reach the tentacle or peduncle of the animal. As nematocysts are pushed proximally and distally from the growth region, because of a rapid multiplication of cells in this region, they become more acid and show an increase in metachromasia. There appears to be an inhibitive substance present in the gastric and growth regions of the hydra which inhibit the full elaboration of the poison within the nematocyst capsule. On the other hand, it is possible that there is a substance, found in the tentacles and peduncle but lacking in the growth and gastric regions, which is necessary to complete the formation of the nematocyst poison. It has been shown that a nematocyst, located in the gastric region of a hydra, when brought under the influence of either the peduncle or tentacle regions of the animal, becomes more acid within a very short time.

It has been concluded that a mature nematocyst is one that contains an acid poison within its capsule. Such a nematocyst occurs only in the tentacular and peduncular regions of a hydra.

BIBLIOGRAPHY.

BOUILLON, J., CASTIAUX, P. and VANDERMEERSSCHE, G. — Structure submicroscopique des cnidocils. *Bull. micr. appl., 8*, 61, 1958.

BRIEN, P. and RENIERS-DECOEN, M. — La croissance, la blastogénèse, l'ovogénèse chez *Hydra fusca* (Pallas). *Bull. Biol. de la France et de la Belgique, 82*, 293-386, 1949.

BURNETT, A. L. — Histophysiology of growth in hydra. *Journ. Exp. Zool., 140*, 281, 1959.

CHAPMAN, G. and TILNEY, L. — Cytological studies on the nematocysts of hydra. I. Desmonemes, Isorhizas, Cnidocils and Supporting Structures. *Journ. Biophys. and Biochm. Cyt., 5*, 69, 1959 a.

CHAPMAN, G. and TILNEY, L. — Cytological studies on the nematocysts of hydra. II. The Stenoteles. *Journ. Biophys. and Biochem. Cyt.*, 5, 79, 1959 b.

KEPNER, W. A., REYNOLDS, B. D., GOLDSTEIN, L. and TAYLOR, J. H. — The structure, development and discharge of the penetrant of *Pelmatohydra oligactis* (Pallas). *Journ. Morph.*, 72, 561-587, 1943.

SEMAL-VAN GANSEN, P. — Le cnidosome de l'Hydre et le bouton urticant. *Bull. Acad. Roy. Belgique, 37*, 650-664, 1951.

SEMAL-VAN GANSEN, P. — La structure des nématocytes de l'hydre d'eau douce. *Bull. Acad. Roy. Belgique, 40*, 269, 1954.

SLAUTTERBACK, D. and FAWCETT, D. — The development of the cnidoblasts of Hydra. An electron microscopy study of cell differentiation. *Journ. Biophys. and Biochem. Cyt., 5*, 441-452, 1959.

WEILL, R. — Contribution à l'étude des Cnidaires et de leurs nématocystes. *Lille : Trav. Stat. Zool. Wimereux, 10-11*, 1, 1934.