THE PHYSIOLOGY OF NEMATOCYSTS[1]

BY

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INTRODUCTION

Grosvenor ('03) has given a brief review of the different theories which have been invented to explain the discharge of nematocysts, and has himself proposed a view which in the present state of our knowledge seems the only one worth careful consideration. Grosvenor's theory is that the discharge of nematocysts in Cœlenterates, and in those animals which derive their nematocysts from them, is brought about by osmotic pressure. His evidence is as follows: Cerata of Eolis immersed in Calberla's fluid, extrude large numbers of undischarged nematocysts. If the fluid is diluted with sea-water, the threads of the capsules are everted. Similar results were obtained when cerata were plunged into "fairly

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strong" solutions of sugar or salt. Here too the nematocysts were not discharged, but when the preparations were subsequently washed out with distilled water, the nematocysts exploded. Grosvenor also experimented with the tentacles of actinians. When these were plucked off, and quickly thrust into a 50 per cent solution of sugar, and teased into small fragments, though many discharged nematocysts were found, pieces in which none had discharged were common enough. Such fragments were isolated and kept for from 24 to 72 hours. The nematocysts remained undischarged until the sugar solution was washed out with water, when approximately 20 per cent of the capsules discharged themselves.

"These facts," says Grosvenor, "seem to show that the discharge of nematocysts is due to osmosis. The capsule apparently contains a solution of such strength that it takes up water from such a weak solution as sea water, but not from the protoplasm of the nematocytes, or the fluids in the alimentary canal of Æolids, or from any of the other solutions mentioned above."

Our interest in the history of the nematocysts of Æolids, ('02;06) led us in the summer of 1908, to undertake a careful investigation of this subject. The work was begun by the Senior author in the Zoölogical Laboratory of the University of Michigan, and was brought to a practical completion in the Marine Biological Laboratory at Woods Hole. To the Director, Prof. F. R. Lillie, we are indebted for the use of a room in the laboratory.

MATERIAL AND METHODS

The material used for the experiments and for the development of methods consisted of Hydra, Metridium, Physalia, and Montagua. Experiments were made with the nematocyst bearing tissues of these animals, and also with the isolated stinging capsules. The methods used for isolating them were peptic and auto-digestion at 35° C., and maceration in sea-water to which crystals of chloretone had been added. In the case of the peptic digestions, all the tissues except the nematocysts, were dissolved in a solution composed of 4 cc. HCl; 1000 cc. H_2O Dist.; and

10 gr. flaked pepsin. The digestive processes took place quite rapidly, in some instances being complete within 24 hours. The solutions were then centrifugated, and there was obtained a thick sediment, composed, in the case of Physalia especially, of countless isolated, undischarged nematocysts. Many discharged ones also were found, but these formed a minority. The same methods were employed in the case of the auto-digestion.

Experiments to be described later, showed that although these methods are adequate, the nematocysts are changed in certain ways by these processes, and we therefore resorted to the maceration method referred to. For this purpose the acontial filaments of Metridium were found extremely good as they are composed of immense numbers of nematocysts held together by a minimum of other tissues. In sea-water to which crystals of chloretone are added slowly from time to time for a period of about 12 hours, the acontia break down, forming a somewhat glutinous mixture in which free undischarged nematocysts occur in great abundance. These were in excellent condition for some of the purposes for which we used them.

In order to store nematocysts for later use, and this was necessary as we secured only one specimen of Physalia, and that early in the summer, we dessicated some of the sediment secured from the centrifuge, preserved some in glycerine, some in sea-water, and some in salt solutions of various concentrations. Under these circumstances the material keeps perfectly well, and can later be used for experimental work.

During the course of the experiments carried out at Wood's Hole it became desirable on several occasions to separate the discharged from the undischarged nematocysts, and also to isolate individual capsules. The former was accomplished in two ways, sometimes by means of what might be called a "capillary filter," the discharged nematocysts failing to be drawn up into a capillary tube just large enough to admit undischarged ones; sometimes by taking advantage of the somewhat lighter specific gravity of the exploded capsules. In a dish containing both discharged and undischarged nematocysts, the former come to lie above the latter and may be completely removed by means of a small glass hook.

Isolation of individual capsules, whenever desirable, was accomplished by means of a capillary tube filled with a suspension of nematocysts. By spreading, from the mouth of such a tube, small drops on a glass slide the nematocysts may be distributed so that each drop contains only a few, or perhaps only one. The drops can then be numbered, and the history of the individual nematocysts followed for any desired length of time. A moist chamber was frequently used to prevent the drops from drying.

EXPERIMENTS WITH MONTAGUA

The nematocysts of Montagua are derived from its prey, Tubularia crocea. The details in this transfer, are being reserved for another paper. For the present purpose it is necessary to know only that these derived nematocysts are stored by certain entodermal cells, the cnidophages, inside the cnidophores of the dorsal cerata, and that when each storage cell has engulfed a certain number it loses its cellular characters and becomes converted, possibly with the assistance of certain neighboring interstitial cells, into a thin transparent bag, the cnidocyst. These loaded cnidocysts lose their connection with the basement membrane to which in earlier stages they are attached, and come to lie free in the lumen of the cnidophore in the distal end of the appendage.

Under certain circumstances the elimination of cnidocysts filled with stinging capsules may be observed under the microscope. If the animal is stimulated mechanically, chemically, or best of all thermally, the extrusion of the cnidocysts takes place. They are shot out of the cnidopores at the tips of the dorsal cerata, not by violent contractions on the part of these appendages, but by unobservable contractions probably of the musculature of the cnidophore. Relaxation of this musculature immediately around the cnidopore is either incomplete, or if complete, is not great enough to allow the easy passage of the cnidocysts. These, while elimination is going on, are often much distorted, but as soon as the pressure from the walls of the cnidopore is relieved, they become spherical. At times they make their appearance as clear bubbles blown by the cnidopore, and they may remain in this condition

until they have been almost completely extruded, when the nematocysts begin to shoot into the visible portion, either one by one, or in groups. When this has happened, the cnidocysts leave their positions at the mouth of the cnidopore, usually on account of the movements of the animal or of its appendages, and may float freely in the water nearby, or may remain adhering to other regions of the ceras.

The nematocysts so extruded, in many instances discharge inside of their enclosures and as their threads penetrate through the wall of the cnidocyst, this may come to resemble a "sperm-bundle," with filaments radiating in all directions. Ultimately the cysts burst, and set free their discharged contents. This, however, is not the usual history—ordinarily the bursting of the cnidocyst and the explosion of its nematocysts take place at the same instant. The questions therefore arise: Why do the cnidocysts burst, and why do the nematocysts discharge? There are involved no living tissues which might be responsible; the nematocysts are not living things, and their enclosing cnidocysts are also dead.

A simple experiment gives the answers. If Montagua is stimulated thermally in a concentrated sugar solution, the elimination of cnidocysts takes place as described, only as soon as they come into contact with the surrounding medium they shrivel. None of the cysts burst, and none of the nematocysts discharge. If now the sugar solution is replaced by distilled water, the cnidocysts swell and burst, and the nematocysts discharge. Discharge, however, is rarely complete; a few nematocysts in every collection of them fail to discharge under circumstances under which the majority explode.

If this experiment is modified, and the elimination is forced to occur in distilled water, the bursting of the cnidocysts and the discharge of the nematocysts take place so quickly, that it is impossible to be more than aware of the processes. Even under these conditions some nematocysts may remain undischarged.

The most plausible explanation of these results is that the bursting of the cnidocysts, and the discharge of the nematocysts are due to absorption of water; that introduction into a medium of higher osmotic pressure than the contents of either the cnidocyst,

or the nematocysts, results in the abstraction of water, and that for this reason, the former shrivel and the latter remain intact. Why some of the nematocysts fail to discharge when the majority explode, will be discussed in connection with later experiments.

EXPERIMENTS WITH THE TENTACLES AND ACONTIA OF METRIDIUM

The results of Grosvenor, and those just described, lend strong support to the idea that the discharge of nematocysts is due to osmosis, and while none of our experiments seem to indicate that this idea is erroneous, the study of the living tentacles and acontia of Metridium, shows that the matter is not quite as simple as might be supposed. In nematocyst-bearing tissues, another factor must be reckoned with, the living nematocyte, the cell which makes the nematocyst and encloses it.

The living tentacles, as well as the acontia of Metridium may be removed without discharging the nematocysts; this can be done very easily in the case of the tentacles, not quite so easily with the acontia, but even in this instance, an abundance of intact threads or pieces of threads, is readily obtained. These can then be treated in various ways, and the behavior of the nematocysts studied.

In certain media, many of the nematocysts leave their natural positions in the mother tissue, but do not discharge; media of much higher osmotic pressure than sea water, may bring about discharge, and heat, electricity and mechanical pressure are effective. At first sight these results seem to be strongly antagonistic to the osmotic theory, but careful analysis of them, either changes all of these data into positive supports or at least disarms them.

In the following table is given a résumé of the details of the experiments on the effects of various media and stimuli on living nematocyst-bearing tissues. The material used is mentioned in the first column; the treatment given it, in the second; whereas the effects on the nematocysts are recorded in the third and fourth columns. The word extrusions is used to designate those instances in which nematocysts, without exploding, left their normal positions in the mother tissue. Such extrusions are due either to

a breaking down of the surface of the tentacles or acontia when exposed to certain media, or in some cases to contraction. All of the experiments were repeated several times, and some, many times, so that the reports are based on the behavior of thousands of nematocysts.

Material used	Treatment	Extrusions	Explosions
Metridium tentacles	sea-water	none	none
acontia	sea-water	none	none
tentacles	distilled water	many	many
acontia	distilled water	many	many
tentacles	saturated sugar solution	none	none
acontia	saturated sugar solution	none	none
tentacles	idem followed by H_2O dist.	many	few
acontia	idem followed by H_2O dist.	all	none
tentacles	saturated sodium chlorid	many	many
acontia	saturated sodium chlorid	few	many
tentacles	Kleinenberg's picro-sulfuric	few	many
acontia	Kleinenberg's picro-sulfuric	none	all
tentacles	sublimate-acetic	few	many
acontia	sublimate-acetic	none	all
tentacles	saturated mercury bichlorid	none	none
acontia	saturated mercury bichlorid	none	none
tentacles	acetic acid	many	many
acontia	acetic acid	none	all
tentacles	hydrochloric acid	few	many
acontia	hydrochloric acid	none	many
tentacles	ammonium hydroxid	few	many
acontia	ammonium hydroxid	none	all
tentacles	95 per cent alcohol	none	many
acontia	95 per cent alcohol	many	many
tentacles	chloroform	none	many
acontia	chloroform	none	many
tentacles	ether	none	many
acontia	ether	many	many
tentacles	chloretone	none	many
acontia	chloretone	many	many
tentacles	mechanical pressure	many	many
acontia	mechanical pressure	many	many
tentacles	heat 100° C.	many	many
acontia	heat 100° C.	many	many
tentacles	heat 0 °C.	none	none
acontia	heat 0° C.	none	none
tentacles	alternating current	many	many
acontia	alternating current	none	all

It is not necessary to give a detailed analysis of the experiments summarized in Table I. In general they indicate that specific chemical effects are not involved, and further that any theory which attempts to explain the discharge of nematocysts, must take account of the nematocyte. This particular phase of the subject, however, can be more profitably discussed after the experiments on isolated nematocysts have been reported. These also will explain some of the above results which at first sight may appear puzzling.

EXPERIMENTS WITH ISOLATED NEMATOCYSTS

A nematocyst is a membranous capsule, one portion of which is prolonged into a thread, ending in a point. In its undischarged state, this thread is introverted, and is stored inside the capsule of which it is an organic part. In addition to the visible filament, the capsule contains certain invisible chemical substances.

On the basis of this knowledge, we may make certain assumptions regarding the causes that bring about eversion of the thread, and these assumed causes can then be tested experimentally. We may assume that in order to bring about discharge, it is necessary to raise the internal pressure of the capsule to a point at which it can overcome the effect due to the uniform external pressure to which the capsule is subject, plus whatever resistance to eversion is offered by the construction of the capsule itself. We may assume further, that the capsule is a membrane, semi-permeable to aqueous solutions, and that it contains substances capable of absorbing water. We may assume also that the membrane is specifically permeable to certain ions, although, if the results of the experiments can be explained without this assumption, postulation of specific permeability becomes unnecessary.

These assumptions were tested experimentally. The results which have been actually obtained appear to be explicable by any one, or any probable combination, of the following factors: increase of internal pressure; decrease of external pressure; reduction in the resistance to eversion due to the construction of the capsule.

Mechanical Pressure

The effect of mechanical distorting pressure was studied by mixing the nematocysts of Physalia or the tentacles of Metridium with granulated salt and grinding the material between glass plates. Sometimes the salt was omitted, and ground glass plates were used. After treatment in this manner, the nematocysts were examined. In those cases in which salt was used, this was dissolved before observations on the results of the treatment could be attempted. In this way a considerable mechanical distorting pressure was applied to the individual capsules, and though the nematocysts of Metridium, on account of their minute size and their delicacy, gave inconclusive results, those of Physalia gave very positive ones. Many partial discharges were obtained. Pressure on the cover glass of a preparation of Physalia nettles also causes many partial discharges. Such pressure as was used in these experiments distorts the capsules, and is effective because the internal pressure of the nematocysts is raised by distortion.

Uniform External Pressure

That the effects of distorting pressure have been correctly interpreted, is clearly shown by the effect of high uniform external pressure. Such pressure was applied by allowing the nematocysts to be drawn up into a capillary tube provided at one end with a reservoir filled with mercury. The open end of the tube was then sealed and the mercury made to expand.

The pressure obtained in this manner, calculated from the contraction of the air bubble inside the tube, and from the bursting strength of the tube, was from 50 to 100 atmospheres. No nematocysts ever discharged when treated in this way.

Solutions

In Table II are presented in condensed form the results of experiments undertaken to discover the effects on isolated nematocysts of the same solutions which had previously been employed on the living tentacles and acontia of Metridium. The isolated

nematocysts of Physalia were not used in this series of experiments for reasons which will become clear later—all the results presented in this section are based on isolated Metridium nematocysts secured by the maceration method.

TABLE II

Solution	Effect
Sea water	none
Distilled water	complete instantaneous discharge
Saturated sugar solution	none
Idem followed by H_2O dist.	partial and slow discharge
Saturated sodium chlorid	none
" strontium chlorid	none
" zinc sulfate	none
" magnesium sulfate	none
" sodium carbonate	none
" potassium carbonate	none
Kleinenberg's picro-sulfuric	complete instantaneous discharge
Sublimate acetic	complete instantaneous discharge
Saturated mercury bichlorid	none
Acetic acid	complete instantaneous discharge
Hydrochloric acid	complete instantaneous discharge
Ammonium hydroxid	complete instantaneous discharge
95 per cent alcohol	none
Chloroform	none
Ether	doubtful
Chloretone	none

In every case, except that of sea-water, dilution occurred when the reagents listed above were brought into the fluid of the suspensions. The error due to this, however, is of no consequence in the present connection. With one or two exceptions, to be discussed later, the same solutions, effective in bringing about the discharge of nematocysts within their mother cells, are capable of causing the same effects when the nematocysts are isolated.

An examination of the table shows that these results give strong support to the osmotic theory. The positive effects of distilled water, of dilute acids, such as Kleinenberg's picro-sulfuric, and sublimate acetic, and the negative results from the use of the saturated solutions of sugar, and of sodium, strontium, magnesium,

and potassium and mercury salts, are all to be expected. Some of the other results, however, require a word of comment.

Alcohol, ether, even if effective, chloroform and chloretone, used because employed in the previous experiments on living tentacles and acontia, bear neither way on the osmotic theory. The action of strong acids and of ammonia remain to be explained. Acids are chemically very active, and it is conceivable that upon penetration into the nematocyst they affect a decomposition of the intracapsular contents, thus increasing the number of molecules present, and hence the internal pressure. Since the H ion is the active one, it is possible that the membrane is specifically permeable to it.

The effect of acids, however, may be explained in the same way as the action of ammonia. The latter is effective possibly on account of its power of disintegrating tissues. If the capsule is weakened at the point where the thread is introverted—a point normally weak—eversion is likely to occur, for as will be shown later, the capsular contents themselves exert a high pressure.

The effect of distilled water on nematocysts which have been treated with a saturated solution of sugar, is due to the fact that sugar probably "gums up" the pores of the capsules. Other agents do the same thing, and it is for this reason that the Physalia material was not used, although in the course of time it would probably have given the same results. This is indicated by the following observations.

In suspensions made in distilled water, from dessicated Physalia nematocysts, as well as from those preserved in glycerine, it was noticed that the older the suspension, the greater the number of completely discharged nematocysts. This increase was so great that in the course of several days the exploded ones began to outnumber those intact. This phenomenon pointed to slow osmotic interchange between the capsular contents and the surrounding medium. Grosvenor, in dealing with pieces of actinian tentacle teased up in a half concentrated sugar solution, found, when the sugar is washed out with distilled water, that "never more than approximately 20 per cent" of the nematocysts discharge themselves. Had he waited, he would no doubt have

found the percentages much higher. The results obtained from the macerated material, in which discharge was complete, and also instantaneous, show that media, such as glycerine, and sugar solutions, either clog the pores, and make diffusion a slow process, or else make the eversion of the thread so difficult that a higher pressure than the normal one is needed to bring about explosion. Both of these causes might be operative together, and, in addition, it must be remembered that the digestive processes in themselves might have the effects suggested, and might also alter the constitution of the intracapsular contents.

Hypertonic Solutions

If the results already described support the osmotic theory, the effect of hypertonic solutions completely demonstrates its correctness. Not only do nematocysts fail to explode in such solutions (Table II) but if left in them for a number of days, they can be made to discharge in media too concentrated to bring about the explosion of normal nematocysts. These results, which will be referred to again in another connection, can be explained only on the assumption that by slow transfusion the intracapsular contents are so changed by the hypertonic solutions, that the nematocysts become able to absorb water from media more concentrated than those toward which they are normally osmotically neutral.

Negative External Pressure

If the explosion of nematocysts is due to pressure from within outward, as the osmotic theory requires, and as the effects of distorting pressure seem to indicate is true, it follows that a negative external pressure might result in explosion, particularly if, as is conceivable, the capsules are in a state of tension. Negative pressure of one atmosphere, produced by suction, gave entirely negative results. This failure, however, is not traceable to a mistake in principle, but to the insufficiency of the negative pressure. The osmotic pressure of sea-water is in the neighborhood of 22 atmospheres (Garrey '04), and as will be shown later, the pres-

sure of the intracapsular fluid must be about the same. It was found that at ordinary temperatures, practically all of the nematocysts discharged in a solution of 70 per cent distilled and 30 per cent sea-water, whereas practically no explosion occurred in a mixture of 60 per cent sea-water and 40 per cent distilled. Since this latter dilution gives $\frac{40}{100} \times 22$, or 9 atmospheres as the minimum pressure required to bring about explosion, it is easy to see why a simple vacuum proved wholly inadequate.

Heat

Low temperatures hinder discharge, and make it necessary to employ solutions of much greater dilution than are needed at ordinary temperatures. High temperatures on the other hand greatly facilitate discharge, and make it possible to explode nematocysts in media more concentrated than sea-water. These effects in all probability are due to a combination of factors.

In the case of low temperatures, the capsule probably contracts, and thus renders more difficult, not only the absorption of water, but the actual extrusion of the thread through the narrow opening out of which it must be everted. The increase in the viscosity of the medium due to the lowering of the temperature is also a considerable quantity. When dealing with high temperatures on the other hand, the viscosity of the surrounding medium is reduced; the expansion of the capsule not only makes absorption easier, but also the actual process of eversion; further the pressure within the capsules must be raised, partly on account of the increased speed of the molecules in the intracapsular fluid, partly on account of an actual increase in the number of molecules present, for Portier and Richet ('02) have shown that the hypnotoxin breaks down at 55° C.

Alternating Current

Although capable of causing the discharge of nematocysts imbedded in their living mother tissues, when applied to the isolated capsules the alternating current proved ineffective. The result is explained by the fact that an alternating current is inca-

pable of changing the concentration of the solution through which it passes, on account of the compensatory effect of the rapid reversals in direction.

RATE OF EXPLOSION

The fact that the rate at which explosion takes place may be greatly modified by treating the capsules with glycerine and sugar, suggested the possibility of controlling the eversion of the thread in other ways. If the osmotic conception is correct, a moderate increase in the concentration of a solution should reduce the speed of the discharge, and a great increase should prevent explosion altogether. Both of these effects were obtained, though under influence of heat, the capsules continued to discharge in media too concentrated to allow explosion at ordinary temperature.

The reduction in the speed obtained by the use of concentrated sea-water, and other media of high osmotic pressure, made possible certain observations on the eversion of the thread which are in complete harmony with the osmotic theory. In such media, when the dilution is just sufficient to bring about explosion, one can see that during the process of eversion, the thread is cast out suddenly, but only to about two-thirds its length. A brief period —less than a second often—of inactivity, due no doubt to the immediate relief of pressure, ensues, and then the remainder of the thread is everted. To observe this effect one must use a medium only a trifle less concentrated than that from which the nematocysts were taken.

VARIATIONS IN THE EXPLOSIVE PRESSURE

In practically all of the experiments on isolated nematocysts, it was noticed that not all of the threads are everted under circumstances under which most of the capsules explode. At certain concentrations no explosions occur; if the solution is diluted, a few incomplete or slow discharges occur; further dilution increases both the number and the rate of the discharges, and finally a point is reached at which the great majority explode. Even here, however, a few remain unaffected unless the medium is

diluted still further. It follows from this that the pressure necessary to explode nematocysts instantaneously varies with the individual capsule, and as these differences occur in nematocysts prepared by the digestion as well as the maceration methods, it is safe to conclude that the observed facts are normal.

It is conceivable that the porosity of the capsular wall may vary with its age, or may vary independently of this, and the same thing is true of the intracapsular contents. Either of these possibilities would account for the facts. It is also true that other slight differences in the construction of the capsules might affect the pressure needed to explode them, and possibly also, not all of them are in equally good working order. In the eversion of a barbed thread, like that characteristic of the nematocysts of Metridium, it would seem that there is ample opportunity for entanglements, capable of being loosened or broken only by increased pressure from within.

APPLICATIONS OF THE OSMOTIC THEORY TO ÆOLIDS AND CŒLENTERATES

Æolids

The experiments described can leave no doubt that osmotic pressure can account for all of the observed facts. The question now arises, why do the nematocysts of nudibranchs discharge on coming into contact with sea-water, whereas those of cœlenterates remain intact?

While enclosed within their mother cells in the cœlenterate, the nematocysts must be osmotically neutral toward their cellular environment; and since they themselves are neutral toward sea-water, it follows that we must consider the nematocyte also osmotically neutral toward its external environment. This neutrality must be disturbed by the sojourn of the nematocysts within the bodies of the nudibranchs, or some other factor must enter to counteract it. After careful consideration of the possibilities that suggest themselves, we have discarded all but one: by slow transfusion, the contents of a nematocyst osmotically neutral toward

sea-water, may be changed, so that it becomes capable of absorbing water, and consequently of raising its internal pressure to the exploding point. To test this idea, isolated nematocysts of Metridium were treated with a saturated salt solution for four days. After this time sea-water, which is osmotically neutral toward freshly isolated nematocysts, was as effective in bringing about discharge as distilled water is when applied to unmodified capsules.

Cœlenterates

A comparison of the results obtained from isolated nematocysts, and from those imbedded in their living mother tissues, suggests that the explanations which hold good for the former class hold equally good for the latter; that in the one case we are dealing with an osmotic interchange directly between the capsule and its surrounding medium; in the other case between the nematocyte and the medium, and that the permeability of the cell to the various reagents used, is such that for practical purposes the nematocyte is non-existent. It must be apparent that in most cases it is impossible to show that this, as a generalization, is incorrect, nevertheless, we believe that it is incorrect, and that the nematocyte, the mother cell of the nematocyst, has something to do with its discharge, possibly not under all circumstances (see Tables I and II) but certainly under some, and perhaps always when the nematocyst is discharged in response to stimuli normal in the lives of cœlenterates.

The efficacy of the nematocyte as a factor in the normal discharge of a nematocyst can be shown in at least three ways. A saturated solution of sodium chlorid is incapable of bringing about the explosion of isolated nematocysts. This, however, is not true when the same solution is applied to the living tentacles and acontia of Metridium. (Table I.) Under this treatment a complete discharge of all the nematocysts occurs. The alternating current, when applied to isolated nematocysts, is ineffective, but when applied to fresh tentacles and acontia, it brings about the discharge of all the nematocysts present.

These two experiments suggest that the cell is effective, and that

the reason why the nematocysts explode under the conditions named, is because the nematocyte is stimulated to do something which brings about discharge. The correctness of this inference can be established, if without destruction, the nematocyte can be eliminated from possible participation in the chain of events. This can be done by narcotization. The most effective agent to use, if used with moderation and care, is chloretone. If the tentacles and acontia of Metridium are narcotized with chloretone, saturated sodium chlorid, 95 per cent alcohol, and chloroform, all of which act as stimuli under normal conditions, do so no longer, and the nematocysts enclosed by their anesthetized mother cells fail to explode. These results seem to point conclusively toward the nematocyte as a factor in the normal discharge of a nematocyst, and this in spite of the fact that distilled water, Kleinenberg's picro-sulfuric acid, sublimate acetic, acetic acid, ammonium hydrate, and ether, are as effective on narcotized material as on normal. All of these liquids are highly penetrating, or contain very penetrating elements, or have specific gravities, so little above that of distilled water, that they act under all circumstances, as though the mother tissues, normal or narcotized, were not there. Heat also is effective when applied to narcotized nematocytes, either because the nematocysts under its influence absorb water, or because their contents break down (p. 373.). In addition to the explanations suggested, it is possible that a nematocyte narcotized sufficiently to be unresponsive to certain stimuli, it is not necessarily sufficiently under anesthesia to render all stimuli ineffective.

Why then do the nematocysts of Cœlenterates explode under normal conditions? Since they are completely enclosed by the fluid contents of the nematocytes, contraction on the part of these cannot be effective, since a uniform external pressure, no matter how high it may be, is incapable of causing discharge. The possibility of a distorting pressure produced by the nematocyte is not absolutely ruled out, though we know of no mechanism by which it might be produced. Appeal to undiscovered cytoplasmic fibrillæ might be made, but with little profit. The osmotic theory on the other hand can be applied here also even if direct evidence is still wanting.

In making an application of the osmotic theory two possible factors suggest themselves, and these, operative singly or together, will account for the facts. It is conceivable that when stimulated, the nematocyte suddenly generates heat; it is also conceivable that the cytoplasm around the nematocyst undergoes chemical and physical changes of such a nature that the capsules are enabled to absorb water, and to raise their internal pressures to the exploding point. Particularly if heat is liberated at the same time that "dilutation" occurs, this theory offers no insurmountable difficulties. The time element need not be considered, for the chemical and physical changes which stimulation sets up in a muscle occur very quickly, and when the proper reduction in the concentration of the surrounding medium has been made, a nematocyst explodes instantaneously.

THE PHYSIOLOGICAL EFFECTS OF NEMATOCYSTS ON OTHER ORGANISMS

On the chemical side, it has been shown by Portier and Richet ('02) that an aqueous extract made from twenty-nine of the tentacles of a Physalia contained enough poison to kill a pigeon within an hour after injection. Curiously enough no inflammation was set up; irritability and temperature were reduced and diarrhœa frequently set in. These experiments, together with the observation that frogs or fish, when placed in contact with the filaments of Physalia, make no attempts to escape, led Portier and Richet to name the poison involved, hypnotoxin. Very little is known regarding its chemical nature. It is destroyed by a temperature of 55° C.; can be precipitated with alcohol; and is non-dialysable. Von Fürth ('03) who gives a résumé of Portier and Richet's work, adds that it is necessary to assume that the nettles, in addition to the hypnotoxin, contain a violent "Reizgift," which accounts for the inflammations, which in spite of the observations quoted above, have been observed in other cases.

On the physical side, the conclusions of Iwanzoff ('96) and the earlier ones of Möbius ('66), are opposed in certain important respects. Iwanzoff states that the physiological effects of the

nettles are due to the numerous poisoned threads which surround and penetrate the victim. Möbius distinctly opposes this view as well as others which are current. For instance he considered the idea that the hairs on the filaments are "back hooks," a mistaken one, not only because in the ripe thread they stand out at right angles, but also because they are too delicate to serve the function attributed to them. Möbius might have added that many types of functional nematocysts are devoid of these barbules.

As for the ability of the thread to penetrate into the tissues of the victim, Möbius considered this impossible, in the first place because the thread "unrolled" too slowly, and in the second place, because its point does not strike the victim. In fact, the point is the last portion of the thread to be everted, and it is, of course, cast out with less force than any other part. The great delicacy of the point was also offered as evidence against the validity of the current belief.

The nettling sensations produced by nematocysts, Möbius did not attribute to the minute punctures made by the filaments, but to the fact that these are saturated with some chemical, which on coming into contact with the skin, produces irritation. This chemical remained undetermined, but relying entirely on microchemical tests, Möbius concluded that it is neither formic acid, nor any other acid.

The existence of hypnotoxin seems to be fairly well established, whereas there is considerable doubt about the inferred "Reizgift." All the phenomena which the "Reizgift" could explain, seem to me to be explicable on the assumption that the filaments, contrary to Möbius' conception of their powers, do actually penetrate the tissues. The idea does not seem to have occurred to him that a thread might penetrate a tissue before being completely everted; he does not seem to have realized at what immense pressures the discharges occur; nor, if he knew of such instances, did he recall that, given sufficient velocity, a stem of hay will shoot through a pane of glass.

Observations, as well as experiments, bearing on the penetrating power of the filaments were made. Thus the acontia of Metridium were placed on fresh tissue taken from a clam, and the

nematocysts were then discharged by fixing the preparation in sublimate acetic. The tissues which had been "shot" in this way were then sectioned, and in several regions it was possible to trace the filaments of the nematocysts through the epidermis into the muscle and connective tissues below. Most of the threads however, did not enter the tissues, but seemed to have been warded off, and in the sections lie tangential to the epidermis. This is what might be expected, for unless a filament penetrates while it is at the height of its speed, it fails to make a puncture at all, for the extreme end is everted too feebly, just as Möbius says.

Direct observations on the behavoir of the nematocysts of Montagua, while still enclosed within the cnidocysts, are much more favorable for the elucidation of this question. We have reported in an earlier section that the nematocysts may explode before the cnidocyst bursts, and that the discharged filaments are capable of penetrating through their enclosing membrane.[2] The length of the discharged filament; the position of the nematocysts inside the cnidocyst, and the diameter of the cnidocyst, make it absolutely impossible for the filaments to penetrate the membrane at any other than the early stages of eversion. The cnidocysts are not large enough to allow anything else; nevertheless the filaments penetrate them, which is exactly what they should do if the osmotic theory of discharge, and the considerations brought forward in the preceding paragraphs, are correct.

SUMMARY

1 The material used consisted chiefly of the living tentacles and acontia of Metridium, and nematocysts, isolated, by digestive and other methods, from Metridium and Physalia.

2 The discharge of nematocysts is due to internal pressure. This pressure may be raised to the exploding point by osmosis and by distortion.

[2] Two months after this paper was written Toppe (Zoologischer Anzeiger Bd. xxxiii, Nos. 24/25) published an account of his very careful observations on the manner in which nematocysts discharge, and showed conclusively that the nettling threads are able to puncture the chitinous covering of a Corethra larva.

3 The explosive pressure varies with the individual nematocysts, and with circumstances. It may be artificially altered. This fact explains why the nematocysts of Æolids explode in seawater, whereas those of Cœlenterates do not unless the nematocyte is stimulated.

4 It is impossible to show that the nematocyte is a factor in the discharge of the nematocysts of Cœlenterates under all circumstances. Nevertheless, this is true under some circumstances, and perhaps always under the conditions which are normal in the lives of cnidaria.

5 The osmotic theory, originally advanced by Grosvenor on very limited evidence, is absolutely supported, as far as isolated nematocysts are concerned, and may be applied to the normal discharge of stinging capsules in Cœlenterates, if we suppose that stimulation of the nematocyte inaugurates changes which result in the liberation of heat or in lowering the concentration of the intra-cellular medium immediately surrounding the nematocyst. Both heat and dilution may be operative.

6 The filaments of nematocysts are capable of penetrating the tissues of other animals, contrary to the opinion of Möbius, but in order to do this, must make their punctures before eversion is complete.

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