The Influences of Immersion Media on the "Longevity" of Isolated Nematocysts of Sea-Anemone[1,2,3]

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In my previous paper (Yanagita, 1943) was reported an efficient method of obtaining isolated preparations of actinian nematocysts in unexploded and workable conditions, together with the results of experiments carried out with these preparations to discuss the mechanism of their explosion. Although the medium in which the isolated nematocysts were kept for the experimentation was almost exclusively 1 M glycerine solution (pH 8.3 by $NaHCO_3$), other sorts of solutions were occasionally tried, different effectiveness being found for different media in preserving the nematocysts in functional state.

It is the object of the present work to determine the last mentioned point in more detail, since informations about the influences of different immersion media upon the nematocysts with respect to the maintenance of exploding capacity were expected to be useful (1) for practical purposes of experimenters working with this material, as well as (2) for theoretical considerations of the structure and working mechanism of the nematocysts.

In addition, the results of measurements of the capsular size before and after the loss of their exploding capacity will be presented, in the hope that it might throw some light upon the causes of the process of inactivation.

Before going further, I wish to express my sincere thanks to the late Professor Takeo Kamada for his original suggestion of the subject. Thanks are also due to Mr. Yukio Hiramoto of the Misaki Marine Biological Station, who kindly helped me in providing research facilities for the performance of the work at the Station.

Material and Methods

An individual of *Diadumene luciae* was placed in a dish of sea water and prodded with a pair of forceps to cause it to throw out its

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3 An abstract of this paper was read before the 22nd Annual Meeting of the Zoölogical Society of Japan, Oct. 11th, 1951.

acontial filaments. It was then hung into a centrifuge tube filled with 1 M glycerine solution (pH 8.3 by $NaHCO_3$) for a period of two minutes to induce the nematocyst extrusion from the acontia. The suspension of isolated nematocysts thus made was immediately centrifuged to obtain a minimum volume of very condensed suspension at the bottom of the tube, so that the suspension medium could be replaced by any one of the experimental media.

The experimental media tested for their effects on the functional longevity of the nematocysts, besides 1 M glycerine solution, were: distilled water, sea water (natural), and M/2 NaCl, M/2 KCl, M/3 $CaCl_2$ and M/3 $MgCl_2$ solutions (each buffered by $NaHCO_3$ to pH 8.3). The effects of each of the solutions were determined by pipetting out from time to time a sample from the nematocysts suspended in that solution and counting the numbers of the exploded and non-exploded nematocysts ("penicilli," Stephenson, 1929) after having added a drop of N/10 HCl solution on a glass slide under a low power of microscope. Explosion per cent. of the total number of nematocysts was plotted against the time of immersion to obtain a curve that may conveniently be called the "aging curve" of nematocysts. Unless otherwise stated, each set of observations was carried out using nematocysts from a single individual of sea-anemone.

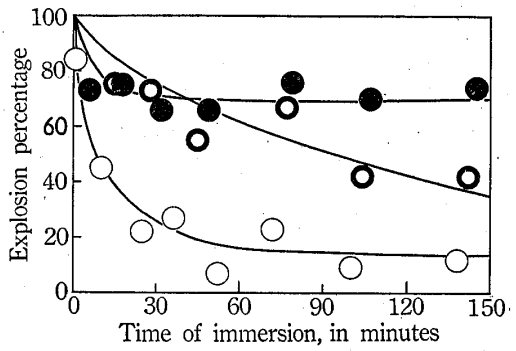

Fig. 1 Aging curves of the nematocysts in 1 M glycerine solution (●) in distilled water (O) and in M/3 $CaCl_2$ solution (○). The three series of observations were carried out in parallel, at least twenty nematocysts being counted for each single plot of the explosion percentage. The curves have been drawn in a rather arbitrary way through the plots; the initial value for explosion percentage was assumed to be 100%. A mixed suspension of nematocysts from two individuals (Nos. 10 and 11) of *Diadumene* was used for this set of aging curves. Temp., 29°C.

Results

The "aging curves" obtained with the nematocysts kept in suspension in the different experimental media appeared to conform to each other in that they primarily are simply declining curves, somewhat similar in form to an exponential curve of unimolecular chemical reaction. The specific effect, if any, of each media upon the nematocysts' aging was to be seen only in the steepness or the "half-value period" of the curve.

1 M glycerine solution (pH 8.3 by $NaHCO_3$) It had already been known in the previous work that glycerine solution was an excellent

preservation medium for the function of *Diadumene* nematocysts. This was confirmed by the present determination, though a rather considerable variation was found, with respect to the steepness of the curves or to the "half-value period" (more than 4 hours), according to the individual from which the nematocysts were derived (see Figures 1 and 2).

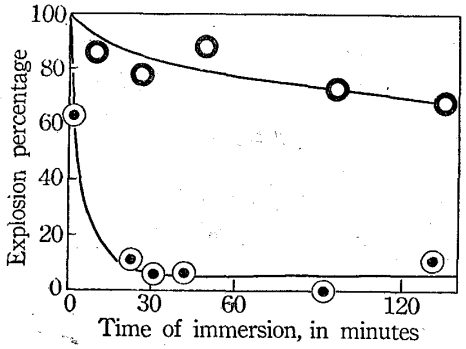

Fig. 2 Aging curves in distilled water (O) and in M/2 NaCl solution (⊙). Nematocysts from the individual No. 3. Temp., 29°C. For the remainder of explanation, see above.

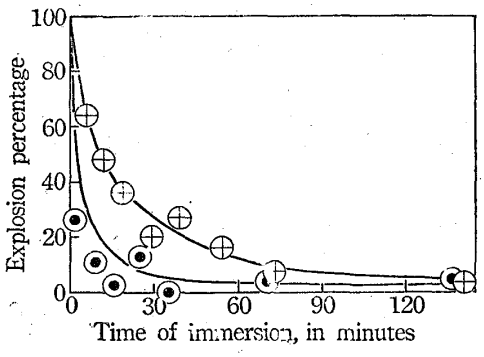

Fig. 3 Aging curves in sea water (⊕) and in M/2 NaCl solution (⊙). Nematocysts from the individual No. 4. Temp., 29°C. For the remainder of explanation, see above.

Distilled water (pH 8.3 by $NaHCO_3$) Pure water, too, was shown to be a suitable medium for preserving the nematocyst activity, but apparently to a less extent than 1 M glycerine solution, thus confirming again the statement in the previous paper (half-value period: 1 to 4 hours; see Figure 1). It is, however, interesting to note that in some cases the nematocysts were so resistant also in this medium that an equal longevity was shown in this and 1 M glycerine soution.

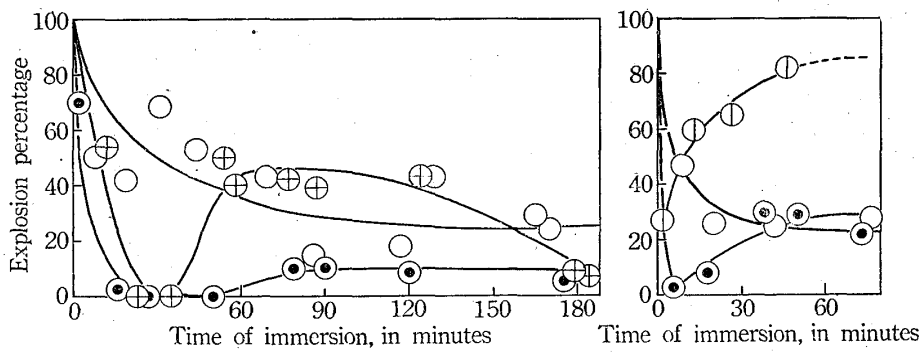

Fig. 4 Left: Aging curves in sea water (⊕), in M/2 NaCl solution (⊙) and in M/3 $CaCl_2$ solution (○). Nematocysts from the individual No. 8. Temp., 29.5°C. Right: Aging curves in M/2 NaCl solution (⊙), in M/2 KCl solution (⦶) and in M/3 $CaCl_2$ solution (○). Nematocysts from the individual No. 9. Temp, 29.5°C. The secondary increase in the explosion percentage are markedly shown in the sea water curve in the diagram on the left and in the NaCl and KCl curves on the right. For the remainder of explanation, see above.

Sea Water (natural), M/2 NaCl and M/2 KCl solutions (pH 8.3 by $NaHCO_3$) Results obtained with these three sorts of solutions may be given under one heading, for they appeared to be very alike. In

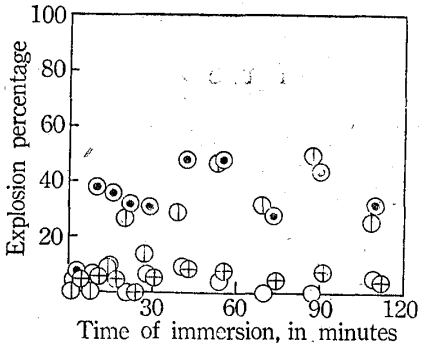

Fig. 5 The percentage of "spontaneous" explosion observed from time to time in nematocyst suspensions in sea water (⊕), in M/2 NaCl (⊙), M/2 KCl (⦶) and M/3 $CaCl_2$ (○) solutions. The observations were run in parallel with the four sorts of suspensions prepared from a single individual (No 12), each suspension being placed in a covered watch glass to enable direct observation under a microscope. Temp., 30°C.

some cases the aging curve for each of these solutions (possibly with the exception of the KCl solution) was a simply declining one, only it dropped very fast, much faster than in the case of distilled water (see Figure 3), while there were other cases in which a peculiar reascent of the curves was recorded (see Figure 4). Though every determination of explosion percentage for these records was made with such a sample of the nematocyst suspension from which practically all the discharged capsules had been drained off previous to the addition of the acid, there were, in such cases, a considerable fraction of nematocysts which had already been discharging in the original suspensions without being acted upon by HCl. In Figure 5 are shown the results of a special series of observations designed to check this fact.

Further experiments would be necessary in order to establish that immersion in these media may really have an effect of recovering, if temporarily, the nematocysts' reactivity to the acid. However, it may safely be said that sea water as well as M/2 NaCl or KCl solution is not only the poorest preservative of the nematocyst activity (half-value period: sea water, about 10 minutes; NaCl, 1 to 3 minutes; KCl, 1 minute or less), but may sometimes an active, though slow-acting, stimulant to explosion.

M/3 $CaCl_2$ solution (pH 8.3 by $NaHCO_3$) This was found to be a rather good preserving medium as compared with NaCl or KCl solution, but a poorer one than distilled water (half-value period: 8 to 15 minutes; see Figures 1, 4, 6 and 7). No case was found with this solution in which either any deviation from a simply declining curve or the "spontaneous" explosion as was mentioned above occurred.

M/3 $MgCl_2$ solution (pH 8.3 by $NaHCO_3$) So far as the results of a limited number of observations go, the aging curve for this medium seemed to be located definitely lower than the curves obtained with M/3 $CaCl_2$ solution, but possibly still above the curves for solutions of the univalent kations (half-value period: about 4 minutes; Figure. 6). On the other hand, one case of the curve showing a secondary rise was also recorded (Fig. 7), and, corresponding to this, evidence of "spontaneous" explosion taking place in this solution was obtained in a check experiment of the type already mentioned.

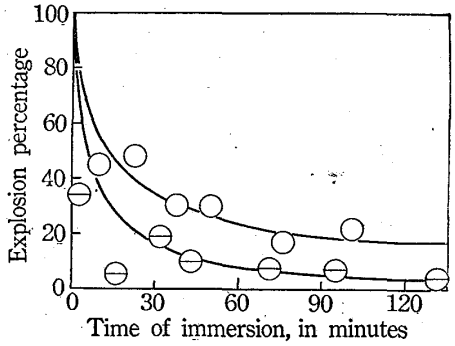

Fig. 6 Aging curves in M/3 $CaCl_2$ solution (○) and in M/3 $MgCl_2$ solution (⊖). Nematocysts from the individual No. 20. Temp., 26°C. Explanation same as before for the remainder.

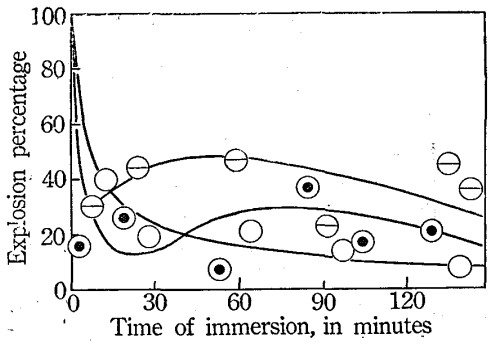

Fig. 7 Aging curves in M/2 NaCl solution (⊙), in M/3 $CaCl_2$ solution (○) and in M/3 $MgCl_2$ solution (⊖). Nematocysts from the individual No. 21. Note the secondary increase in explosion percentage shown by the NaCl and $MgCl_2$ curves. Temp., 26°C. Explanation same as before for the remainder.

Considerations

It was in distilled water that the active process of extrusion of nematocysts from *Diadumene* acontia has originally been found to take place. This finding was soon followed by another that the presence of glycerine in a concentration of 1 mol (i.e., nearly isotonic to sea water) does not reduce at all the effectiveness of distilled water in causing the extrusion (Yanagita, 1943). Although the nature of, as well as the immediate cause for, the phenomenon of extrusion remains unknown as yet, it is interesting to note that the same media that are effective in inducing extrusion were now shown to be most effective also in keeping the extruded nematocysts in their capacity of function.

It is further shown from the results given above that addition of any one of the electrolytes so far tested will reduce more or less markedly the longevity of freed nematocysts. Now the functional longevity of the nematocysts as they remain in their natural seat in the mother cells (cnidoblasts) or in the acontial tissue can reasonably be taken as infinite, at any rate within the range of time dealt with in the present study. The present type of observations might therefore throw light on the chemical nature of the immediate medium in which the organelles find themselves in vivo. It may thus be suggested that the nematocysts are embedded in the acontial tissue with the whole or a certain part of their capsular surface protected in some way from the direct contact with the electrolytes.

Will (1919) confirmed a considerable volume reduction of the unexploded nematocysts of *Hydra* and of *Syncoryne* after having been immersed overnight in their "natural" media, though he did not inquire into the relation of this phenomenon with the disappearance of exploding capacity. In the course of the present study, an attempt was made

to determine the capsular size before and after the loss of exploding capacity by means of an eyepiece micrometer. For each single set of determinations, fifty unexploded penicilli of *Diadumene* were measured for their length, as they were in 1 M glycerine solution still with a sufficiently high rate of explosion, and, then, another fifty of penicilli sampled from the same suspension were measured in M/3 $CaCl_2$ solution after the complete loss of exploding capacity, So far the determinations are rather insufficient in number, but significant values were found in most sets of determinations for a size reduction of the capsules which had suffered aging in the $CaCl_2$ solution.[1]

It might be suggested from this result that a decrease in the intracapsular pressure due, say, to a leakage or permeability increase of the capsular wall is the essential process involved in the "aging", and that the influences of media on the nematocysts' aging consist in retarding or accelerating the progress of such a change, at least in the case of $CaCl_2$ medium. Whether this may be the case or not, the action of electrolytes on the nematocysts in accelerating their aging is very likely to be a surface one, since they were apparently shown by the aging curves to be so rapid that a penetration of substance through the capsular wall is hardly conceivable in the mechanism of action. This view may further be strengthened by referring to the impermeability of the capsular wall to water (Yanagita, 1943), and to the keratin nature of the capsular wall substance which was recently reported to be probable in the case of the nematocysts of *Corynactis viridis* (Brown, 1950). However, any discussion as to the form of the aging curves would be inappropriate on the basis of the material which is available at present.

Finally, another finding in the course of the present study is that sea water, as well as pure solution of NaCl, KCl or $MgCl_2$ in concentrations isotonic to sea water, will often induce explosion of the freed nematocysts in a rather long interval of time (10 to 15 minutes). This delayed action of salines is in a marked contrast to the immediate action found with acids and alkalis, and suggests a mode of action different from the latter cases, and possibly involving a factor of permeability somewhere in the chain of events.

Summary

1. The explosion per cent. of the total number of nematocysts was counted after applying N/10 HCl solution to a sample which was taken from time to time from a nematocyst suspension prepared from the

[1] It may be mentioned in this connexion that by addition of form-aldehyde (about 7 per cent. in 1 M glycerine solution) the nematocysts were found to be deprived of their power of explosion in a matter of moment, and that this was accompanied by an evident diminution of the capsular volume.

acontia of *Diadumene luciae*, and the influences of different sorts of suspension media upon the nematocysts' longevity were studied on the explosion percentage-time curves ("aging curves").

2. The best preserving media for the function of isolated nematocysts proved to be distilled water and 1 M glycerine solution.

3. Sea water, as well as pure solutions of its component electrolytes in concentrations isotonic to sea water, was found to accelerate the aging of nematocysts. In NaCl or KCl solution the nematocysts lost their exploding capacity faster than in $CaCl_2$ solution.

4. Delayed actions of KCl, NaCl and $MgCl_2$ solutions on nematocysts in causing their explosion was observed.

5. A way in which the present type of observations may contribute to the elucidation of the chemical nature of the milieu in vivo for nematocysts was suggested.

6. A volume reduction of the nematocysts accompanying their aging was shown to be probable, leading to a suggestion as to the nature of the influences of the media upon the longevity of nematocysts.

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