Discharge-Inducing Concentrations of Acids and Bases for the Nematocysts of Sea-Anemone[1)]

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The acids and bases have long been known as agents of a general applicability to evoke the discharge of nematocysts, through the observations of early workers (e.g., Hadzi, 1909, and Ewald, 1916, in Hydrozoa; Weill, 1925, in actinians). One of the present authors reported also that the nematocysts ("penicillum" type) isolated from the acontia of *Diadumene Luciae*[2)] can be caused to discharge by applying sufficient amounts of acid or of base (Yanagita, 1943). So, a N/10 HCl solution was used in the course of his subsequent work (Yanagita, 1951) for testing the discharging capacity of the isolated nematocysts which had been allowed to age in various kinds of media. So far, however, the limit of concentration of acids or bases for inducing the discharge has not been determined. It is the object of the present study to carry out such determinations and to make some considerations of the nature of the discharge-inducing action of acids and bases on the basis of those determinations.

The authors wish to acknowledge their indebtedness to the director, Dr. I. Tomiyama, and the staff of the Misaki Marine Biological Station, where facilities were provided for the present investigation. Their particular thanks are due to Mr. Y. Hiramoto for his kind help in many ways throughout their staying at the Station.

Material and Method

The resting nematocysts were isolated from the acontia of *Diadumene* by hanging the animal into a centrifuge tube filled with 1 M solution of glycerine, just in a similar manner as in the previous work. Since 1 M glycerine solution had already been found to be the medium most favourable for preserving the nematocysts' capacity of discharging (Yanagita, 1951), the nematocysts were allowed to stay in suspension in the glycerine solution throughout the course of experiment. The test for the discharge-inducing effect of an acid or alkali solution was made

1) An abstract of this paper was read before the 24th Annual Meeting of the Zoological Society of Japan, at Sendai, October 4th, 1952.

2) The specific trivial, referred to as "*luciae*" in the previous papers, will be written henceforth with the initial letter in capital, following the way of Dr. Uchida (1932), who first identified the Japanese species with the present name.

using a drop of the nematocyst suspension which was pipetted out from the stock suspension and placed on a glass slide under the microscope. Then, the superfluous liquid was removed by means of a finely drawn pipette (leaving about 0.03 cc. of the liquid surrounding the nematocysts) and a drop (about 0.06 cc.) of the test solution was added. The discharge percentage for each successive test was obtained by counting the numbers of discharged and undischarged nematocysts, in the same way as in the previous study and using at least thirty nematocysts.

There were some proportion of such nematocysts which came to be broken down instead of being normally exploded by the action of acids or bases, especially in the cases of stronger concentrations. They were included in the numbers of "discharged".

Two sorts of regents were always tested in combination (acid-base, acid-acid, or base-base), each in a series of dilutions, making use of a nematocyst suspension prepared from a single specimen of *Diadumene*. In order to be sure that the tests for the two reagents were made with the nematocysts at a comparable stage of aging, the two were always tested by turns. Further, such a double series of dilutions for the pair of regents was runned twice in a close succession to complete a single set of tests, so that an aging effect of the nematocysts, if any, could easily be detected.

Each set of tests thus made with a single individual of *Diadumene* gave four of such curves (two for each of the two reagents tested) in which the explosion percentage were plotted against the normality values of dilutions (see Figure 1). These may conveniently be called "normality-explosion percentage curves". From these, the "pH-explosion percentage curves" were derived, not by direct pH determinations of the test solutions, but by means of the pH data for the acid and alkali solutions available from the literature (Britton, 1932, and Yoshimura, 1940).

Rather considerable amounts of errors were found to accompany the percentage reading of each test, notably about the 50% level. They seemed largely due to (1) the mode of applying the experimental solutions to the nematocysts, a uniform and instantaneous exposure of all the nematocysts to the solutions being not to be expected, and (2) the way of estimating percentage values from the rather limited numbers of nematocysts. However, this did not much prevent the position of the normality-explosion percentage curve as a whole from being determined with a fair definiteness.

Results and Discussions

The following sorts of acids and bases were tested for their discharge-inducing effect, each in a series of dilutions: HCl, H_2SO_4, citric acid (strong or fairly strong acids) ; acetic acid (weak acid) ; KOH, NaOH (strong bases) ; NH_4OH (weak base).

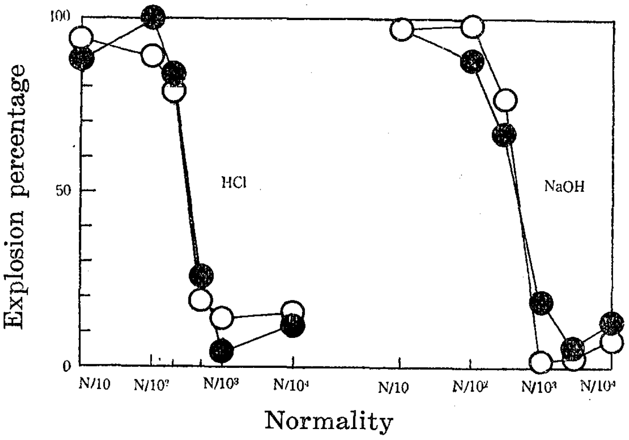

Fig. 1. Normality-explosion percentage curves as recorded simultaneously for HCl and for NaOH: a complete record from a representative set of tests (No. 2) carried out with penicilli from a single specimen of *Diadumene*. The circles and dots indicate the readings from the first run and those from the second run of tests, respectively. Temp. 28.4~28.6°C.

It was just as might have been expected that the pH-explosion percentage curves turned out to be of a sigmoid form for all the acids and bases which were tested (see Figures 1 and 2). When a logarithmic scale was taken for the abscissae (i.e., normality values), the curves showed a fairly sharp inflexion in the middle, so that it was easy to find graphically the normality value corresponding to 50% explosion for any acid or base, by means of interpolation.

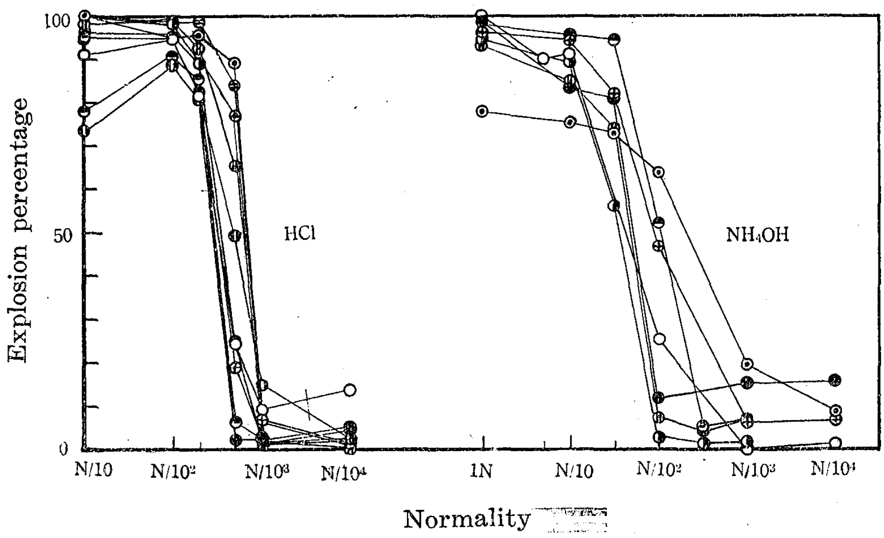

Fig. 2. Ten normality-explosion percentage curves for HCl and seven ones for NH_4OH, presented in superposition. Each of the superposed curves comes from an individual *Diadumene*, and gives the means of the readings from the first and the second runs of tests. Temp. 26~30°C.

First, it must be noticed that the aging of nematocysts which had to be in progress during the course of experimemts (taking seventy minutes or so for a complete set of tests) seemed to be such as offer no appreciable interference with the validity of the present determinations, since there were no appreciable differences found between two curves obtained for the first and the second times with the same nematocyst suspension and with the same reagent (Figure 1).

It will further be seen from Figure 2 that the curve for any sort of acid or of base was fairly well reproducible from individual to individual from which the nematocysts were taken. This reproducibility extends further to the cases of comparison between the curves obtained with different sorts of acids or bases, so long as these all belong to the so-called strong acids or the strong bases. Moreover, the curves for the strong acids and those for the strong bases appeared to be nearly symmetric in form to each other.

On the other hand, there were appreciable divergences to be found between the normality-explosion percentage curves obtained with the strong acids and those obtained with the weak acid (acetic), as well as between the curves for the strong bases and those for the weak base (NH_4OH); the curves are appreciably shifted toward the side of higher normality values in the cases of the weak acid or the weak base, as compared with the cases of the strong acids or strong bases, respectively (Figure 3).

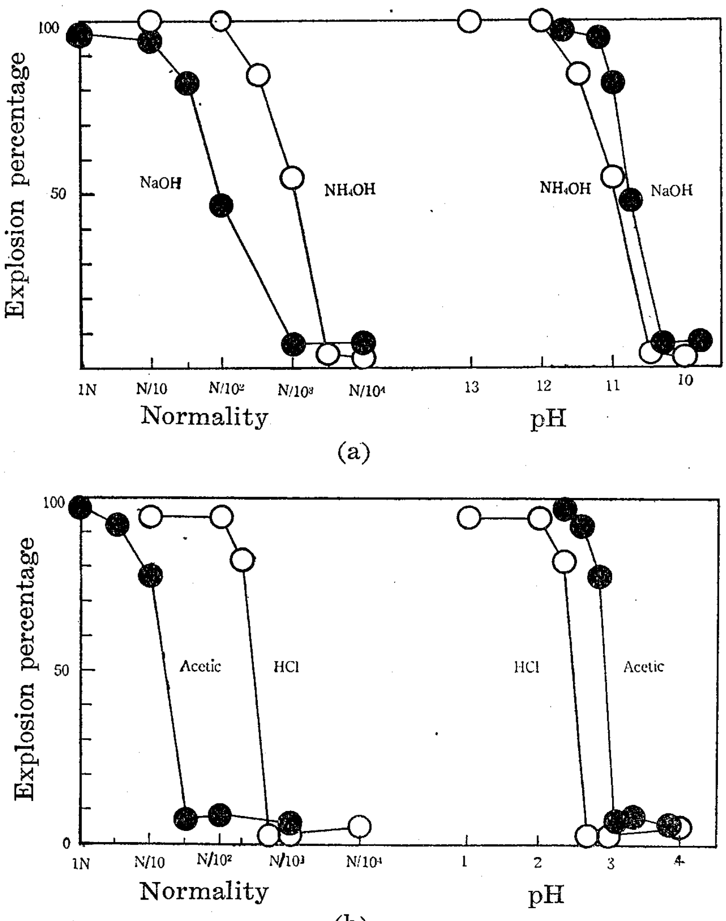

Fig. 3. Normality-explosion percentage curves as compared with the corresponding pH-explosion percentage curves.

(a) NaOH and NH_4OH, as recorded in combination in a single set of tests (No. 12). Temp. 26.0~27.2°C;

(b) HCl, from a set of tests (No. 10), and acetic acid, from another set of tests (No. 35). Temp. 27~30°C.

Such differences in the position and form of the curves are abolished almost altogether when we next take the pH-explosion percentage curves (see Figure 3). Here, not only the curves for the strong acids or bases, but the curves for all the acids or bases, be they strong or weak, were found to nearly coincide with each other.

This fact suggests that the discharge-inducing effects of acids and bases directly depend upon the pH values of the solutions rather than upon the total molecular concentrations (i.e. normality), the unionized residues or the likes. Such a consequence might seem somewhat peculiar when we recall that weak acids and bases, through their higher power of permeation, are known to have a specific effectiveness in so many kinds of physiological processes (see, e.g., Höber, 1926, p. 500, and Heilbrunn, 1937, p. 116). The present results, which seem to permit the effects of acid and alkali solutions to be explained simply in terms of their pH values, may instead indicate that anything like a factor of membrane permeability is not involved in their actions in question.

Such a conclusion is indeed rather in favour of the hypothesis as to the discharge mechanism of *Diadumene* nematocysts already proposed by one of the authors (Yanagita, 1943). Namely, according to that hypothesis, some mechanism which is acting against a pre-existing intracapsular pressure to prevent the eversion of the stinging thread in the resting nematocysts, should be directly attacked from outside by acid or alkali, with the result of its destruction, and this constitutes the event of nematocyst discharge. The present point of view may well be in accord with the interesting theory presented more recently by Kepner et al. (1943 ; 1951), since they admit, in the case of actinian nematocysts, that the larger, proximal segment of the thread ("bottle-brush" of Stephenson, 1929) be just literally everted at the time of discharge, though the smaller "terminal thread" should be formed as a gelation product from liquefied capsular contents ejected out of the thread tip.

The data obtained in the present study may be summarized as in Table I, which gives the normality as well as pH values found graphically for each acid or base as corresponding to 50% explosion, together with the mean pH values for all the acids and for all the bases, respectively. It will be seen from the table that the pH values for the 50% explosion are fairly constant irrespective of the sorts of acids or of bases, while there are considerable fluctuation in the normality values for the same percentage of explosion.

Figure 4 shows a schematic representation of all these results, on which the following statement may reasonably be based: So far as the acids and bases here studied are concerned, the penicilli of *Diadumene Luciae* should be able to remain resting only at the pH values of the medium ranging from 2.9 to 11.0. (The range must be somewhat narrowed from both the upper and the lower sides if we consider the dilution (to about two thirds) of the acid and alkali solutions by the small amounts of glycerine solution leaft surrounding the nematocysts.)

Table I

Acids or Bases	Numbers of determinations	Mean normality values for 50% explosion (n)		$-\log n$	Mean pH values for 50% explosion
		$\times 10^{-4}$N			
H_2SO_4	3	9.7	(8~13)*)	3.0 (2.9~3.1)	3.0 (2.9~3.1)
Citric	3	14	(10~16)	2.9 (2.8~3.0)	3.3 (3.2~3.4)
HCl	10	25	(16~32)	2.6 (2.5~2.8)	2.6 (2.5~2.9)
Acetic	5	1260	(800~1800)	0.9 (1.1~0.7)	2.8 (2.7~2.9)
				Mean:	2.9
KOH	4	11	(8~14)	3.0 (2.9~3.1)	11.0 (10.9~11.2)
NaOH	10	24	(8~50)	2.6 (2.3~3.1)	11.3 (10.9~11.7)
NH_4OH	7	163	(50~280)	1.8 (1.6~2.3)	10.8 (10.5~10.9)
				Mean:	11.0

*) Figures in the parenthesis indicate the minimum and maximum values obtained.

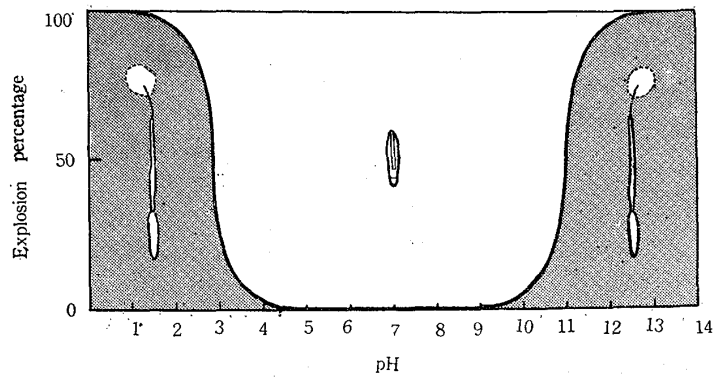

Fig. 4. A diagram to show schematically the overall results. The unshadowed area of the diagram corresponds to the range of conditions in which the penicilli of *Diadumene* will remain undischarged.

That is to say, the "plug-mechanism's" capacity to suppress the thread eversion could only be maintained within that pH range, some of its physical properties being altered at once and just in a similar way at pH's both lying above and beneath these limits.

Summary

1. Discharge-inducing effects of some acids and bases were observed in different concentrations with the penicilli of *Diadumene Luciae*, and the limit of dilution to be effective was determined for each sort of acid and of base.

2. The curve relating the explosion percentage to the normality values of the experimental solutions was found to be of a sigmoid character for any acid or base here dealt with. Differences in position and form of these curves which were to be found between different sorts of acids or bases, respectively, almost vanished when we turn to

the "pH-explosion percentage curves".

3. Conclusion was reached that the discharge-inducing effect of acids and bases may depend simply upon the hydrogen or hydroxyl ions, respectively, which act upon the capsules from outside. That conclusion was shown to be in accord with the hypothesis of "plug-mechanism" formerly presented by one of the authors.

4. The pH values expected to elicit the discharge in fifty per cent. of *Diadumene* penicilli were shown to be 2.9 for the acid side and 11.0 for the alkaline side.

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