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Discharge responses of the nematocysts of the stinging nettle Chrysaora quinquecirrha to external stimuli

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DISCHARGE RESPONSES OF THE NEMATOCYSTS OF THE STINGING, NETTLE CHRYSAORA QUINQUECIRRHA, TO EXTERNAL STIMULI

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DISCHARGE RESPONSES OF THE NEMATOCYSTS OF THE STINGING NETTLE, CHRYSAORA QUINQUECIRRHA, TO EXTERNAL STIMULI

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ABSTRACT

Chrysaora quinquecirrha nematocysts *in situ* and isolated in suspension were systematically exposed to a wide range of chemical and mechanical stimuli to determine which ones would initiate discharge.

Nematocysts *in situ* were found to be highly responsive to stimuli and showed well-marked, immediate discharge when treated with acid, base and electrical stimuli. Slight discharge occurred with salt solutions, beef extract, beef extract and contact, human hair and change in temperature. Discharge increased with the concentration of the reagent or the strength of the mechanical stimuli. A significant increase in discharge occurred in isolated nematocysts treated with HCl, acetic acid and sodium hydroxide. The amount of discharge was found to increase with time.

In comparing *in situ* nematocysts to isolated ones it was found that the former show a greater sensitivity to stimuli. They respond to a larger number of stimuli, they respond faster and at lower concentrations than do isolated nematocysts. This difference seems to indicate that the presence of the cnidoblast is essential for immediate discharge and that a cellular process is involved.

In situ nematocyst discharge in response to food or

contact appears to involve a two-fold stimulus, chemical and mechanical, as has been suggested by other investigators.

INTRODUCTION

Chrysaora quinquecirrha, the stinging nettle, which is present in large numbers in the Chesapeake Bay and other coastal areas during the summer months, is well-known to inhabitants and vacationers because of the irritation inflicted by its nematocysts.

Only a small amount of information has been published on the factors that cause the discharge of nematocysts in the Cnidaria. Most research was concerned with the isolation and identification of the associated toxin. Factors initiating discharge are in most cases given only superficial treatment. Moreover, in prior research C. quinquecirrha has not been used in the study of nematocyst discharge.

Pantin (1942), who worked with Anemonia sulcata, presents a thorough review of prior work on nematocyst discharge. Jones (1947) subjected hydras to a wide range of chemical and mechanical stimuli and, like Pantin, concludes that activation of the discharge mechanism involves a two-fold stimulus, chemical and mechanical. The chemical stimulus first decreases the surface tension, thereby lowering the threshold of resistance of the cnidoblast to physical stimuli which, transmitted by the cnidocil, cause discharge.

Yanagita and Wada (1954 and 1959) and Yanagita (1959, 1960a and 1960b) conducted a series of experiments on the

sea anemone, Diadumene luciae, which showed that nematocysts in situ on the acontial filament are more responsive to chemical and mechanical stimulation than are isolated ones. Blanquet's (1970) work on the sea anemone Aiptasia pallida, agrees with the work of Jones (1947), Pantin (1942), and Yanagita (1960a and 1960b). Furthermore it indicates that pH has an effect on the discharge of nematocysts in A. pallida.

In view of the limited research on nematocyst discharge in C. quinquecirrha, the present work was undertaken. This investigation involves the responses of in situ and isolated nematocysts of C. quinquecirrha to a variety of mechanical and chemical stimuli.

MATERIALS AND METHODS

Stinging nettles were collected during the summers of 1970 and 1971 in the Chesapeake Bay near Deltaville, Virginia (sal. approx. 1.5%) and brought to the University of Richmond for study.

Nematocysts In Situ

Nettles were collected and put into one-gallon wide mouth jars and returned alive to the laboratory where they were placed in large enamel dish pans that contained water from the area in which they were collected. Nettles were stored in the laboratory for a period not exceeding five days. Preliminary work showed that nettles retained longer than five days suffered tentacle deterioration. Deterioration

of the tentacle resulted in spontaneous discharge of the nematocysts and decreased the reaction time to stimuli. Pantin (1942) observed the same reactions with A. sulcata. Tentacles were cut from the nettle and placed in a culture dish that contained sea water. Individual tentacles were then transferred with forceps from the dish to a glass slide. Chemical or mechanical stimuli were applied and each tentacle was observed for five minutes with a stereo microscope to determine if discharge took place. Chemical reagents were applied to tentacles with a pipette. Mechanical, electrical and temperature stimuli were applied to tentacles bathed in sea water. Each stimulus was repeated twenty times.

The procedure for determining discharge followed the methods of Yanagita (1960a and 1960b) and Pantin (1942). Discharge was determined by a visual examination and ranked as follows: (-) no discharge; (+) scattered discharge along tentacle in the range of 1-25 discharges; (++) 26-100 discharges; (+++) large indeterminate number of discharges; (++++) rapid, total discharge of all nematocysts. Total discharge is the discharge of all nematocysts that would discharge with a particular stimulus and the application of additional reagent caused no further discharge.

Isolated Nematocysts

Undischarged nematocysts, free of the cnidoblast and cellular debris were obtained by following the procedure of Rice and Powell (1970). In order to obtain large

quantities of free nematocysts 1 gal jars were packed with C. quinquecirrha and allowed to autolyze in the refigerator at 5 C from one to two weeks. This material was strained through Marquisette Nylon Netting (400 openings/ sq. inch) and then through Swiss bolting cloth (No. 12, 125 mesh). The screened suspension was placed in a refigerator (5 C) and allowed to settle for 24 hrs, after which the supernatant was decanted and discarded. The residue that contained the nematocysts and cellular debris was centrifuged at 7000 rpm (6000g) for 15 min. The supernatant was discarded and an equal volume of boiled, filtered sea water from the collecting area was added to the residue. The centrifuge tubes were gently shaken to loosen the cellular debris overlaying the nematocysts, which stuck firmly to the bottom of the tubes. The process was repeated three times with the loosened cellular debris being poured off each time. The residue was thoroughly mixed with sea water (1.5%), centrifuged at 7000 rpm for 15 min, and the supernatant decanted. The process of washing was repeated until a mass consisting almost entirely of undischarged nematocysts was obtained. This suspension was stored at 5 C or frozen and was used over a period of six months.

Experiments were conducted on the nematocysts by setting up two test tubes each containing 2 ml of the nematocyst suspension and 2 ml of reagent. Control groups consisted of 2 ml of nematocyst suspension and 2 ml of sea water. Test tubes were shaken to mix the contents. A hemocytometer was

used to count discharged and undischarged nematocysts after exposure to each reagent (Burnett et al, 1968). Counting was done at intervals of 1 hr, 24 hrs, 30 days and 60 days. Between counts the test tubes containing suspensions were plugged with cotton and kept at 5 C. Percentages of discharged versus undischarged nematocysts were subjected to a two-factor analysis of variance (Weiner, 1971). The two factors under study were concentration (C) and time (T) with repeated measures along the time dimension. In the event of significant interaction (C x T), a posteriori tests (Newman-Keuls) were performed to determine specific effects of the various concentrations. Further, experimental groups were compared to control groups with the Newman-Keuls test of ordered means (Weiner, 1971). The .05 level of confidence was chosen for all statistical tests performed in this study. Results are given in tables 11-36 and figures 1-6.

All reagents with the exception of the salt solutions were prepared with boiled, filtered sea water (1.5% sal.) from the collection area (Yanagita, 1960a). The term sea water used throughout this paper refers to water from the collection area. The salt solutions were prepared with distilled water. All solutions were prepared using a volume to volume ratio. The food extract solutions were made from raw food sources. Beef extract was prepared from ground beef; fish extract from Leiostomus xanthurus (spot); crab extract from Callinectes sapidus Rathbun (blue crab). To obtain these extracts the material was allowed to stand in

the refigerator for 24 hrs. It was then filtered and the extract kept in the refigerator between experiments to prevent spoiling. Human saliva was also used in some experiments.

RESULTS

Nematocysts In Situ

Nematocysts in situ are highly responsive to treatments with a wide range of reagents (Tables 1-10). Discharge of nematocysts increased with the concentration of the reagent or the strength of the mechanical stimuli. The strength of the reagent, weak acid (acetic, NH_4Cl), strong acid (HCl) and strong base (NaOH), as reflected in the pH readings, also had an effect on the amount of discharge.

Sodium Chloride - No discharge occurred in the NaCl solutions until the 10% concentration was reached (Table 1). Discharge of nematocysts occurred on initial contact with the solution. Additional reagent did not produce further discharge. The 25% concentration produced similar results. At the 35% concentration discharge also occurred only on initial contact but the number of discharged nematocysts was greater (Table 1).

Hydrochloric Acid - Discharge of nematocysts occurred at all concentrations of HCl and increased as the concentrations increased. Response was minimal at concentrations of .05% and .1%. A time delay of approximately 5 minutes occurred before discharge began in both concentrations (Table 2). Discharge at these concentrations was not total as an

addition of a stronger HCl (5%) solution produced additional discharge. Rapid, total discharge occurred with .5%, 1% and 5% concentrations (Table 2). There was a 30 second time delay before discharge began and it continued for approximately 2 minutes.

Acetic Acid - No discharge occurred at the .05% concentration. The .1% concentration caused minimal discharge with a time delay of 5 to 10 minutes in the initiation of discharge (Table 3). A large indeterminate number of nematocysts discharged at the .3% concentration with a time delay of only 30-60 seconds. Rapid, total discharge took place immediately in concentrations of .5% to 5% (Table 3).

Ammonium Chloride - No discharge occurred with the weak acid NH_4Cl until a 5% concentration was reached. Discharge increased from (++) to (+++) as the concentrations were increased from 5% to 20% (Table 4). A 5% concentration of acetic acid was applied to the tentacles and produced further discharge indicating that the NH_4Cl concentrations did not produce total discharge.

Sodium Hydroxide - The treatment of tentacles with the strong base NaOH caused a large number of discharges even at the .5% and 1% concentrations. Rapid, total discharge occurred at all concentrations from 2% to 20% (Table 5).

Food Extracts - Only the beef extract caused a discharge of nematocysts (Table 6). The amount of discharge was minimal and occurred only as the beef extract passed along the tentacle.

Food Extracts + Contact - This experiment was conducted to observe the reaction of a single tentacle to a chemical and a mechanical stimulus. The same food extracts were used as in the preceding experiment, but at the time of application of the extract the tentacle was stroked along its surface with a metal probe. Discharges occurred only with the beef extract plus contact (Table 7). The beef extract alone caused minimal discharge (+). A greater number of discharges (++) occurred when the metal probe was rubbed along the tentacle surface covered with beef extract.

Electrical Shock - A physiograph equipped with platinum needle electrodes was employed to apply electrical shocks from 0.1 to 130 volts. The electrodes were placed 1 cm apart on living tentacles in sea water. The frequency of shocks was 2/sec. with a M/S of 0.5. No discharge occurred with shocks from 0.1 to 3 v (Table 8). Minimal discharge (+) occurred at 5 v and increased to 25 to 100 discharges (++) at the 10 v level. Large amounts of discharge (+++) occurred from 25 to 130 v (Table 8). Total discharge was not achieved even at 130 v since chemical treatment of the tentacle with a 5% acetic acid solution caused additional discharge. The pattern of discharge with electrical shock was unique. With the 5 and 10 v shocks the greatest amount of discharge occurred directly on either side of the electrodes and then continued between the electrodes. As each new shock passed through the tentacle, additional nematocysts discharged. After 4 or 5 shocks all discharge ceased. When the voltage was increased

from 25 to 130, greater amounts of discharge occurred over the entire tentacle rather than only between the electrodes.

Contact in Sea Water - Mechanical stimuli applied to tentacles in sea water included a glass bead, human hair, a metal probe, paper and other tentacles. Of these stimuli only the root of a human hair touched to the tentacle caused discharge and it was minimal (Table 9). The shaft of the hair had no effect.

Temperature - Sea water at the designated temperatures (Table 10) was applied to a moist tentacle on a slide under the microscope. No discharge occurred at temperatures below 25 C; minimal discharge occurred at 80 C; and a substantial discharge was observed at 100 C.

Nematocysts in Suspension

A statistical analysis of the experiments are shown in Tables 11-36 and Figures 1-6.

When subjected to a two-way Analysis of Variance, only two experiments, acetic acid (Table 12) and sodium hydroxide (Table 19), produced significant discharge.

The four concentrations of acetic acid showed no significant difference across all time periods (Table 12). However, there was a significant difference between the four time periods collapsed across all concentrations. Also there was significant interaction between concentration and time, producing greater nematocyst discharge. Significant difference in discharge occurred across the time periods from 1 hr to 60 days with the 2.5% and 5% acetic acid

concentrations (Table 13). No significant change occurred over the time periods with the 10% and 50% concentrations. Figure 1 shows the significant increase in discharge across time for the 2.5% concentration. A significant increase in discharge occurred with the 5% concentration at 1 hr, 24 hrs and 30 day time periods, but the 60 day discharge value is not significantly different from the 10% and 50% concentrations (Figure 1). The 10% and 50% concentrations of acetic acid show random fluctuations with no significant difference across time periods (Figure 1).

Nematocyst suspensions treated with sodium hydroxide also showed significant increases in discharge. Sodium hydroxide (Table 19) produced significant differences in discharge between the concentrations across all time periods. There was also a significant increase in discharge between the three time periods collapsed across all concentrations. A significant interaction of concentration and time also is indicated. A tremendous increase in discharge occurred at the 5% and 10% NaOH concentrations (Table 20). In comparison, the concentrations from .25% to 2.5% give no significant difference in discharge (Table 20). The table of means (Table 18) and the graph of the means (Figure 4) indicate that the lower concentrations vary only slightly among themselves as well as across time periods.

Experiments using hydrochloric acid (Table 15), salt solutions (Table 17), ammonium chloride (Table 22) and food extracts (Table 24) indicated no significant difference in

nematocyst discharge due to concentration, time or the interaction of concentration and time.

A comparison of all six experimental groups to their controls showed that only acetic acid, hydrochloric acid and sodium hydroxide produced a significant increase in nematocyst discharge above the control groups. No significant increase in discharge was produced with ammonium chloride (Tables 33, 34), salt solutions (Tables 29, 30) or food extracts (Tables 35, 36). The nature of these reagents, their concentrations and the duration of time in contact with the nematocysts had no effect on increasing discharge.

The Newman-Keuls test of ordered means conducted on acetic acid (Tables 25, 26) shows that acetic acid produced a significant increase in nematocyst discharge above the control group. The mean for the control group (C = 75.00) is significantly smaller than means for 5%, 10% and 50% acetic acid concentrations (Table 25). There is no significant difference between the control and the 2.5% group. The four acetic acid concentrations do not show increased discharge with higher concentrations. Thus increasing the concentration of acetic acid above 5% had no significant effect on increasing discharge. However, increased discharge above the control occurred at a concentration as low as 5%. In comparing the control group against the acetic acid groups across the four time periods, the Newman-Keuls test shows that the control mean is significantly smaller than means for all four time periods (Table 26). Therefore, nematocysts treated with

acetic acid showed increased discharge with time, whereas the control did not. The mean for the 30 day time period is significantly larger than the mean for the 1 hr time period indicating increased discharge with time from 1 hr to 30 days, after which discharge levels off (Table 26).

Hydrochloric acid experiments as indicated in the ANOV (Table 15) show no significant difference in discharge among concentrations or time periods nor any interaction. This is also indicated in the Newman-Keuls test. The mean for the control group is significantly smaller than the means for all four concentrations and all three time periods (Tables 27, 28). Thus addition of HCl increased discharge above the control, but the increase in concentration above 2.5% and increase in time from 1 hr to 30 days did not increase discharge.

Comparison of sodium hydroxide experimental means to their control mean of 40.0 shows large increases in nematocyst discharge. No significant difference occurs between the control mean and the means for the experimental groups from concentrations of .25% to 2.5% NaOH. Concentrations from .25% to 2.5% also show no significant increase in discharge among themselves. However, large significant increases in discharge occur at the 5% and 10% concentrations (Table 31). Sodium hydroxide concentrations did not effect discharge significantly until a 5% concentration was reached. The 5% concentration also differed significantly from the lower concentrations and the 10% concentration produced significantly greater discharge

than the 5% concentration (Table 31). Comparison of the control to NaOH concentrations across time periods of 1 hr, 24 hrs and 30 days shows that the control mean is significantly smaller than the 24 hr and 30 day means, but is not significantly different from the 1 hr mean (Table 32). The 1 hr mean is significantly smaller than the 24 hr and 30 day means, and the 24 hr mean is significantly smaller than the 30 day mean (Table 32). This indicates that discharge increased as the time of exposure to NaOH increased from 1 hr to 30 days.

Comparing the response of nematocysts *in situ* with nematocyst suspensions to the reagents used, several differences can be seen. *In situ* nematocysts were more responsive to the addition of a reagent. A discharge response occurred immediately or within 5 to 10 minutes after addition. The response of nematocysts in suspension was slower taking at least 1 hr and in some cases up to 30 days before significant discharge occurred. Furthermore, the *in situ* nematocysts also discharged with a much lower concentration. The discharge reaction was similar in both cases in as much as increase in concentration and time increased the amount of discharge.

DISCUSSION

This investigation is the first of its kind performed with *C. quinquecirrha* nematocysts, both *in situ* and isolated in suspension, in which the nematocysts were systematically

exposed to a wide range of stimuli to determine which would initiate nematocyst discharge. The studies of other researchers using organisms such as Hydra, Physalia and several different sea anemones have produced information on nematocyst discharge both in agreement and disagreement with one another.

The discharge of nematocysts in situ on the tentacle when treated with dilute solutions of acid and base has been observed in Anemonia sulcata (Pantin, 1942), Physalia and Metridium (Parker & van Alstyne, 1932) and Diadumene luciae (Yanagita & Wada, 1954). In agreement with their findings this investigation also shows that dilute solutions of acid (.05% HCl, .1% acetic acid and 5% ammonium chloride) and the base NaOH (.5%) produce in situ discharge of C. quinquecirrha nematocysts (Tables 2,3,4, and 5). A time delay before the initiation of discharge which occurred when the tentacles were treated with dilute HCl and acetic acid (Tables 2,3) is not recorded in the earlier investigations. This delay is probably the time required for these weak acid concentrations to mix with the sea water clinging to the tentacle and to come in contact with the cnidoblasts. Parker and van Alstyne (1932) observed that 10% concentrations of HCl, acetic acid and NaOH caused the rapid discharge of Physalia and Metridium nematocysts. In support of their findings the present work also shows that a 10% concentration of these reagents caused rapid discharge of nematocysts.

Yanagita and Wada (1954) working with Diadumene luciae and Blanquet (1970) working with the sea anemone Aiptasia pallida observed that pH played a role in nematocyst discharge. Yanagita and Wada (1954) set the pH limit for acids at 3.0 and bases at 11.0. Between these limits little or no discharge of in situ nematocysts occurred. Blanquet's findings are in general agreement with Yanagita and Wada. Blanquet, (1970) however, worked with both in situ and isolated nematocysts. His pH limits for in situ nematocysts are 4 and 11. Outside of this range extensive discharge occurs. With isolated nematocysts Blanquet found decreased sensitivity to pH induced discharge. His pH limits for isolated nematocysts are 2.0 - 11.0 (Blanquet, 1970). The results of acetic and hydrochloric acid experiments on in situ nematocysts support the findings of Yanagita and Wada, and Blanquet. Acetic acid results (Table 3) are an excellent example. The .05% concentration (pH 3.6) produced no discharge, the .1% concentration (pH 3.5) a slight amount and the .3% concentration (pH 3.0) a large amount of discharge. Discharge appeared to begin somewhere between pH 3.0 - 4.0 and increase as the pH decreased. The weak acid ammonium chloride produced discharge of in situ nematocysts at pH 6.4 and 6.1 (Table 4). This is in complete disagreement with the findings of Yanagita and Wada, and Blanquet. It is difficult to present an explanation for this. However, since different organisms were used, this could be a possible explanation for the disagreement. Further work dealing exclusively with pH values and their relationship to discharge would perhaps clarify the disagreement. For bases,

Yanagita and Wada (1954) and Blanquet (1970) agree that below pH 11.0 little or no discharge occurs. The findings of this study fall well within that limit and support their investigations (Table 5). Regarding the responses of isolated nematocysts to acids and bases this study agrees with Blanquet (1970) that isolated nematocysts are less sensitive to stimuli which normally evoke their discharge *in situ*. This study agrees with Blanquet's limits of pH 2.0 - 11.0 for isolated nematocysts. Significant discharge occurred with acetic acid and HCl at pH readings close to 2.0 or below (Tables 11, 14). Ammonium chloride with pH from 6.0 to 6.7 (Table 21) produced no significant discharge, which is in agreement with Blanquet. Discharge did occur with the base NaOH at pH 10.1 and up (Table 18) but the increase appears to be related to time as well as pH. As time increased up to 30 days the NaOH treated nematocysts showed complete structural disintegration. This study with isolated *C. quinquecirrha* nematocysts goes beyond the function of pH in discharge to also include time and the interaction of time and concentration, therefore it is somewhat difficult to compare results with those of Blanquet (1970) except superficially.

Phillips and Abbott (1957) state that a weak acid or base will totally discharge the isolated nematocysts of *Metridium senile* in a 12 to 18 hr time period. Isolated *C. quinquecirrha* nematocysts treated with weak acid and base did show increased discharge with time. However, even at a 30 day time period total discharge was never observed (Tables

11, 14, 18 and 21).

C. quinquecirrha nematocysts, in situ and isolated, showed little or no response to distilled water or salt solutions of varying concentrations (Tables 1, 16 and 17). Blanquet (1970) obtained similar results with A. pallida nematocysts treated with salt solutions. Jones (1947) found that whole Hydra would discharge nematocysts when placed in distilled water. Phillips and Abbott (1957) claim that suspended nematocysts of Metridium senile would totally discharge in 12 to 18 hrs when placed in distilled water. This is contrary to the findings of the present investigation (Tables 16, 17) and also that of Rice and Powell (1970) using C. quinquecirrha.

The localized discharge of in situ nematocysts by electric current (Table 8) is in agreement with the findings of Pantin (1942) and Yanagita (1960a).

Studies of various researchers using food extracts, contact, and food extracts plus contact as stimuli have produced a variety of results. Food extracts did not produce well-marked discharges in the studies of Pantin (1942) using A. sulcata and Parker and van Alstyne (1932) using Physalia and Metridium. In this study only beef extract by itself or accompanied with contact caused in situ nematocysts to discharge (Tables 6, 7). Isolated nematocysts treated with food extracts showed no increase in discharge above the control group (Table 23). Pantin (1942) observed that a 50% saliva solution caused A. sulcata to discharge nematocysts. Greater

discharge occurred if the saliva solution was followed by contact with a glass bead. Yanagita (1960a) also observed D. luciae to discharge with saliva plus contact. C. quinquecirrha showed no response to saliva either in solution or combined with contact (Tables 6, 7). An interesting observation made by Pantin (1942), Yanagita (1960a) and also this study is that human hair causes nematocyst discharge. In this investigation discharge was produced with the hair only when the bulb of the hair came in contact with the tentacle. The hair itself did not cause discharge. Discharge appears to be due to a chemical stimulus produced by the wet bulb that had just been pulled from the follicle. Contact with objects that are of no food value such as sand grains, glass beads and probes produced no discharge (Table 9, Pantin, 1942, and Yanagita, 1960a). The overall conclusion that can be reached from all observations is that the presence of food does cause nematocyst discharge if it is simultaneous with or followed by contact. The discharge mechanism in A. sulcata, C. quinquecirrha and D. luciae with reference to food seems to indicate a two-fold stimulus, chemical plus mechanical.

Several conclusions can be reached from the data of this investigation and those of other researchers. First, since the findings of the studies mentioned here are so similar it can be concluded that nematocyst function and mechanism of discharge are similar in all organisms tested. Second, the observations of this study with C. quinquecirrha point to a two-fold discharge mechanism for in situ nematocysts as

suggested by Pantin (1942) for A. sulcata and Jones (1947) for Hydra. A chemical and mechanical stimulus is necessary to initiate nematocyst discharge in response to food or contact (Tables 6,7 and 8). The discharge that occurs in response to strong acid, base, electrical, salt and temperature stimuli is the result of the violent and harsh actions of these stimuli, which may lead to cell membrane disorganization or destruction and the subsequent disintegration of the capsule wall leading to discharge. These stimuli are not natural and do not follow the two-fold mechanism necessary for a natural discharge of nematocysts. Third, in situ nematocysts have a greater sensitivity to stimuli than isolated nematocysts. In situ nematocysts respond to a larger number of stimuli, they respond faster and at lower concentrations than do isolated nematocysts. This is in agreement with Yanagita (1960a) and Blanquet (1970). This difference in the response of in situ and isolated nematocysts seems to indicate that the presence of the cnidoblast is essential for natural discharge and that a cellular process is involved in the discharge of C. quinquecirrha nematocysts. Blanquet (1970) reached a similar conclusion. Just what the discharge process involves is not clear. Further work with the cnidoblast and the cell membrane may provide the answer.

SUMMARY

1. *Chrysaora quinquecirrha* nematocysts both *in situ* and isolated in suspension were subjected to a wide range of chemical and mechanical stimuli.

2. Well-marked, immediate discharge occurred with *in situ* nematocysts treated with acid, base and electrical stimuli. Slight discharge occurred with salt solutions, beef extract, beef extract plus contact, human hair and temperature.

3. Isolated nematocysts showed well-marked discharge with hydrochloric acid, acetic acid and sodium hydroxide. The amount of discharge was found to increase with time.

4. *In situ* nematocysts are more responsive to stimuli than isolated nematocysts.

5. *C. quinquecirrha* data collected in this study is similar to those collected for *Hydra*, *Physalia*, *Metridium*, *D. luciae*, *A. sulcata* and *A. pallida*. This suggests a similarity of the function and mechanism of discharge.

6. Discharge of *C. quinquecirrha* nematocysts *in situ* appears to be a two-fold mechanism involving chemical and mechanical stimuli.

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TABLE 1. - Discharge data for *in situ* nematocysts treated with salt solutions. (-) no discharge; (+) scattered discharge along tentacle in the range of 1-25 nematocysts; (++) in the range of 26-100 discharges; (+++) large indeterminate number of discharges; (++++) rapid, total discharge of all nematocysts that would discharge.

Concentration	pH	Amount of discharge	
Distilled water	5.4	(-)	
.5% NaCl	6.4	(-)	
1% NaCl	6.5	(-)	
1.5% NaCl	6.2	(-)	
2% NaCl	6.4	(-)	
5% NaCl	6.5	(-)	
10% NaCl	6.2	(+)	Only on initial addition of reagent
25% NaCl	6.0	(+)	On initial addition of reagent
35% NaCl	5.9	(++)	On initial addition of reagent

TABLE 2. - Discharge data for *in situ* nematocysts treated with hydrochloric acid (HCl). See legend in Table 1.

Concentration	pH	Amount of discharge
.05% HCl	2.3	(+) Time delay 5 mins.
.1% HCl	2.1	(++) Time delay 5 mins.
.5% HCl	1.6	(++++)
1% HCl	1.4	(++++)
5% HCl	1.1	(++++)

TABLE 3. - Discharge data for *in situ* nematocysts treated with acetic acid. See legend in Table 1.

Concentration	pH	Amount of discharge
.05% Acetic acid	3.6	(-)
.1%	3.5	(+) Time delay 5-10 mins.
.3%	3.0	(+++) Time delay 30-60 sec.
.5%	2.9	(++++)
1%	2.6	(++++)
2%	2.5	(++++)
3%	2.4	(++++)
5%	2.2	(++++)

TABLE 4. - Discharge data for *in situ* nematocysts treated with ammonium chloride (NH_4Cl). See legend in Table 1.

Concentration	pH	Amount of discharge
.5% NH_4Cl	7.4	(-)
1%	7.2	(-)
2%	6.5	(-)
5%	6.4	(++)
10%	6.1	(+++)
20%	6.1	(+++)

TABLE 5. - Discharge data for *in situ* nematocysts treated with sodium hydroxide (NaOH). See legend in Table 1.

Concentration	pH	Amount of discharge
.5% NaOH	11.7	(+++)
1%	11.8	(+++)
2%	11.6	(++++)
5%	11.3	(++++)
10%		(++++)
20%		(++++)

TABLE 6. - Discharge data for *in situ* nematocysts treated with food extracts. See legend in Table 1.

Concentration	pH	Amount of discharge
Beef 20%	5.4	(+)
Blue crab 20% (*Callinectes sapidus* Rathbun)	7.3	(-)
Fish 20% *Leiostomus xanthurus*	6.6	(-)
Human saliva 50%	7.0	(-)

TABLE 7. - Discharge data for *in situ* nematocysts treated with food extracts plus contact with a metal probe. See legend in Table 1.

Concentration	pH	Amount of discharge
Beef 20%	5.4	(++)
Blue crab 20%	7.3	(-)
Fish 20%	6.6	(-)
Human saliva 50%	7.0	(-)

TABLE 8. - Discharge data for *in situ* nematocysts treated with electrical shock. See legend in Table 1.

Volts	pH	Amount of discharge
0.1	7.8	(-)
1	7.8	(-)
3	7.8	(-)
5	7.8	(+)
10	7.8	(++)
25	7.8	(+++)
50	7.8	(+++)
100	7.8	(+++)
130	7.8	(+++)

TABLE 9. - Discharge data for *in situ* nematocysts exposed to contact in sea water. See legend in Table 1.

Stimulus	pH	Amount of discharge
Glass bead	7.8	(-)
Human hair	7.8	(+)
Metal probe	7.8	(-)
Paper	7.8	(-)
Tentacles	7.8	(-)

TABLE 10. - Discharge data for *in situ* nematocysts treated with a rapid decrease and increase in temperature. See legend in Table 1.

Temperature C	pH	Amount of discharge
0	7.8	(-)
10	7.8	(-)
20	7.8	(-)
35	7.8	(-)
50	7.8	(-)
80	7.8	(+)
100	7.8	(+++)

TABLE 11. - Means of discharge data for nematocysts in suspension treated with acetic acid

ACETIC ACID		TABLE OF MEANS				
Concentration (C)	pH	Time periods (T)				
		1hr	24hrs	30 days	60 days	total
2.5%	2.2	78.0	89.75	96.25	111.25	93.81
5%	2.1	94.75	123.75	117.0	94.75	107.56
10%	2.0	99.75	107.0	122.75	100.0	107.38
50%	1.5	110.75	92.25	117.0	100.25	105.06
	Total	95.81	103.19	113.25	101.56	103.45

FIGURE 1. - Graph of means for nematocysts in suspension treated with acetic acid

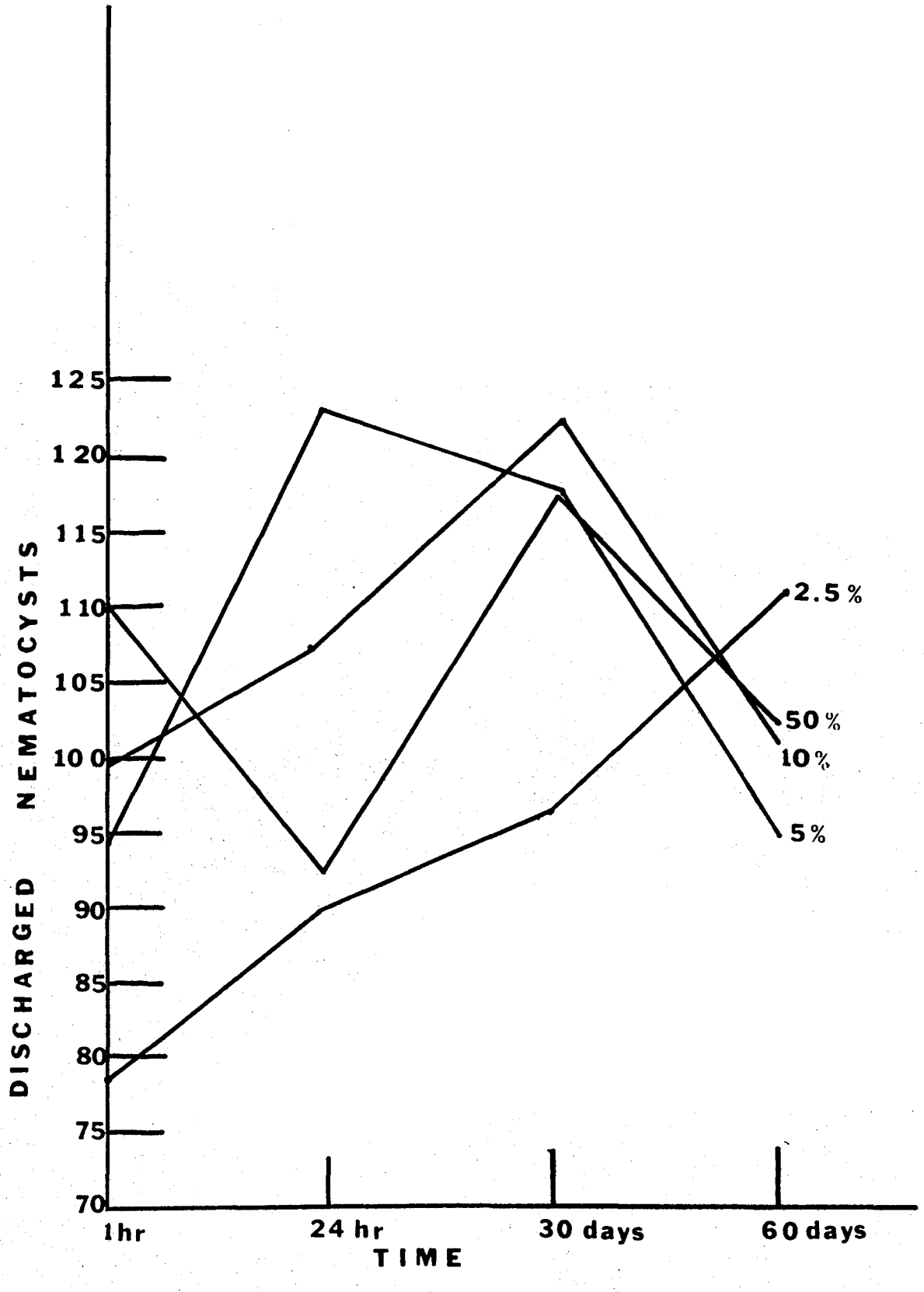
DISCHARGED NEMATOCYSTS
125
120
115
110
105
100
95
90
85
80
75
70
2.5%
50%
10%
5%
1hr
24 hr
30 days
60 days
TIME

TABLE 12. - Analysis of Variance for discharge data for nematocysts in suspension treated with acetic acid

Analysis of Variance

Source of Variation	d.f.	MS	F
Between subjects	15	786.40	
concentration (C)	3	681.60	.84
error (C)	12	812.61	
Within subjects	48	308.58	
time (T)	3	842.68	4.37 *
C x T	9	593.41	3.08 *
error (T)	36	192.86	

$F_{.95}(3,12) = 3.49$

$F_{.95}(9,36) = 2.17$

$F_{.95}(3,36) = 2.89$

TABLE 13. - Analysis of variance of discharge data for nematocysts in suspension treated with acetic acid

Analysis of Variance			
Source	d.f.	MS	F
Time at 2.5% (C)	3	768.73	3.99 *
Time at 5% (C)	3	905.90	4.70 *
Time at 10% (C)	3	465.42	2.41
Time at 50% (C)	3	482.90	2.50
Error	36	192.86	

$F_{.95\ (3,36)} = 2.89$

TABLE 14. - Means of discharge data for nematocysts in suspension treated with hydrochloric acid.

HYDROCHLORIC ACID		TABLE OF MEANS			
Concentration (C)	pH	Time periods (T)			
		1hr	24hrs	30 days	total
2.5%	1.3	84.8	100.3	103.1	96.1
5%	1.1	99.1	111.5	99.8	103.6
10%	.9	98.1	97.1	120.2	106.1
50%	.5	110.5	106.0	98.8	105.1
	Total	98.3	103.8	106.0	102.2

FIGURE 2. - Graph of means for nematocysts in suspension treated with hydrochloric acid

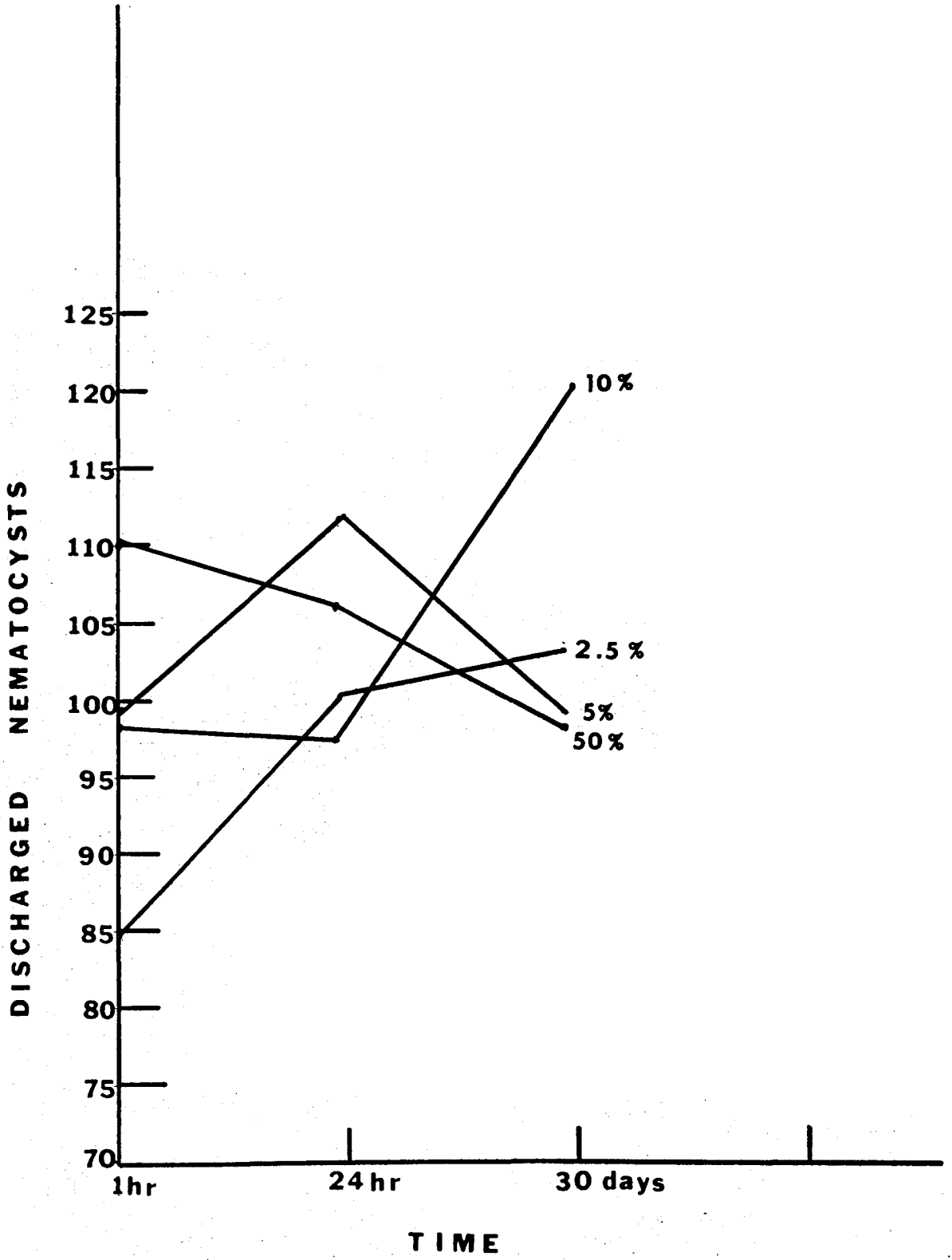
DISCHARGED NEMATOCYSTS
125
120
115
110
105
100
95
90
85
80
75
70
10%
2.5%
5%
50%
1hr
24hr
30 days
TIME

TABLE 15. - Analysis of variance for discharge data for nematocysts in suspension treated with hydrochloric acid

Analysis of Variance			
Source of Variation	d.f.	MS	F
Between subjects	15	326.94	
concentration (C)	3	242.35	.70
error (C)	12	348.09	
Within subjects	32	451.79	
time (T)	2	254.34	.53
C x T	6	420.33	.88
error (T)	24	476.11	

$F_{.95\ (3,12)} = 3.49$

$F_{.95\ (2,24)} = 3.40$

$F_{.95\ (6,24)} = 2.51$

TABLE 16. - Means of discharge data for nematocysts in suspension treated with salt solutions

SALT SOLUTIONS		TABLE OF MEANS				
Concentration (C)	pH	Time periods (T)				
		1hr	24hrs	30 days	60 days	total
Distilled water	6.4	101.3	85.3	102.0	94.8	95.8
.5%	6.5	99.5	90.5	97.5	83.8	92.8
1%	6.5	86.0	89.0	99.3	89.8	91.0
2.5%	6.7	88.8	73.0	68.0	81.3	77.8
5%	6.5	102.3	103.5	72.8	66.8	86.3
12.5%	6.4	83.0	71.5	67.8	69.0	72.8
17.5%	6.3	58.3	60.0	72.8	68.3	64.8
	total	88.4	81.5	82.9	79.1	83.0

FIGURE 3. - Graph of means for nematocysts in suspension treated with salt solutions

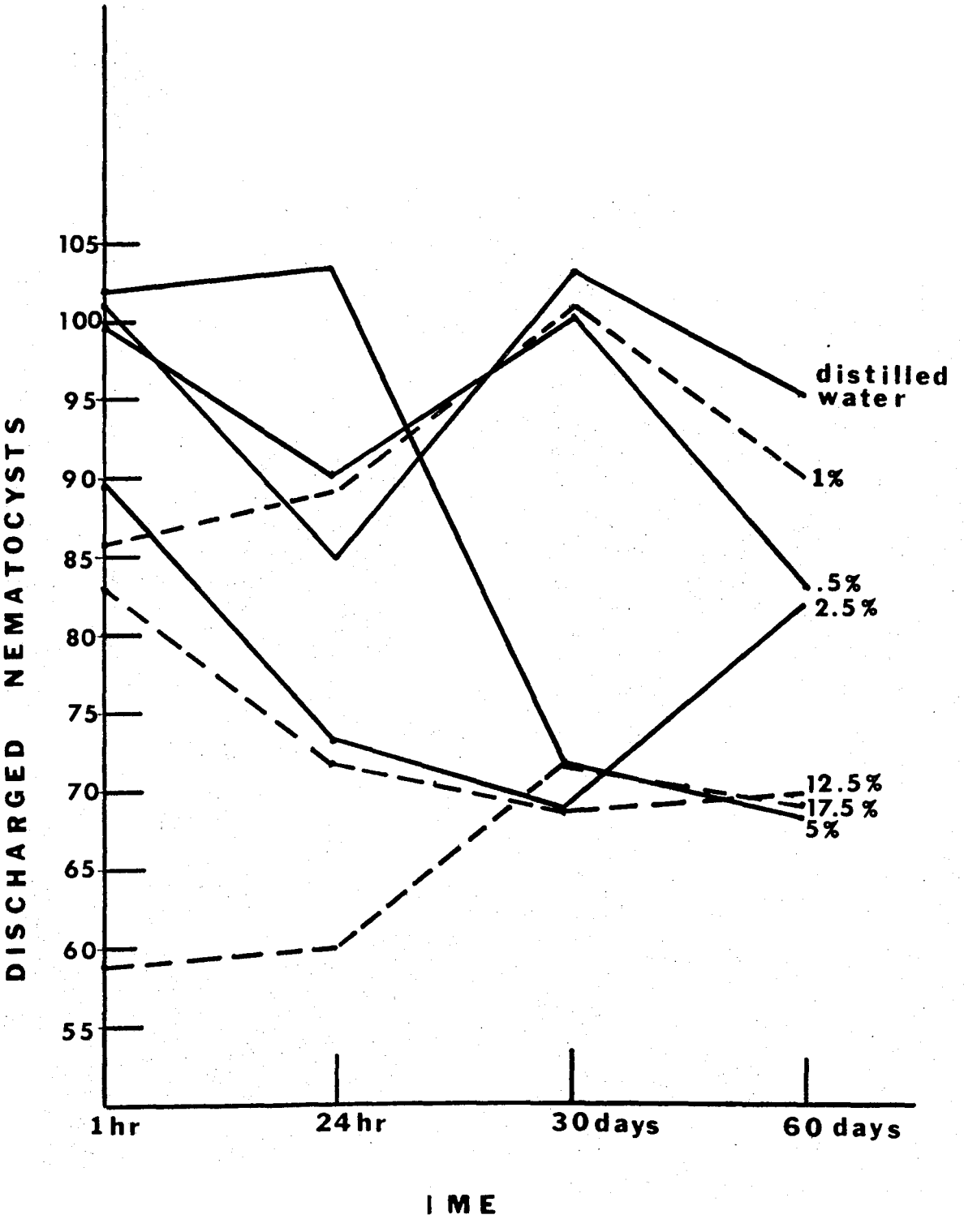
DISCHARGED NEMATOCYSTS
105
100
95
90
85
80
75
70
65
60
55
1hr
24hr
30days
60 days
IME
distilled water
1%
.5%
2.5%
12.5%
17.5%
5%

TABLE 17. - Analysis of variance for discharge data for nematocysts in suspension treated with salt solutions

Analysis of Variance			
Source of Variation	d.f.	MS	F
Between subjects	27	1237.50	
concentration (C)	6	2126.77	2.16
error (C)	21	983.43	
Within subjects	84	402.69	
time (T)	3	432.18	1.07
C x T	18	391.75	.97
error (T)	63	404.42	

$F_{.95}(6,21) = 2.58$

$F_{.95}(3,63) = 2.76$

$F_{.95}(18,63) = 1.80$

TABLE 18. - Means of discharge data for nematocysts in suspension treated with sodium hydroxide

SODIUM HYDROXIDE		TABLE OF MEANS			
Concentration (C)	pH	Time periods (T)			
		1hr	24hrs	30 days	total
.25%	9.2	48.2	43.0	59.5	50.3
.5%	9.7	38.2	32.3	42.5	37.7
1%	10.1	35.7	42.5	95.8	58.0
2.5%	11.6	33.2	64.0	107.0	68.08
5%	11.2	38.8	143.5	1000.0	394.09
10%		140.8	604.2	1000.0	581.66
	total	55.8	154.9	384.1	198.3

FIGURE 4. - Graph of means for nematocysts in suspension treated with sodium hydroxide

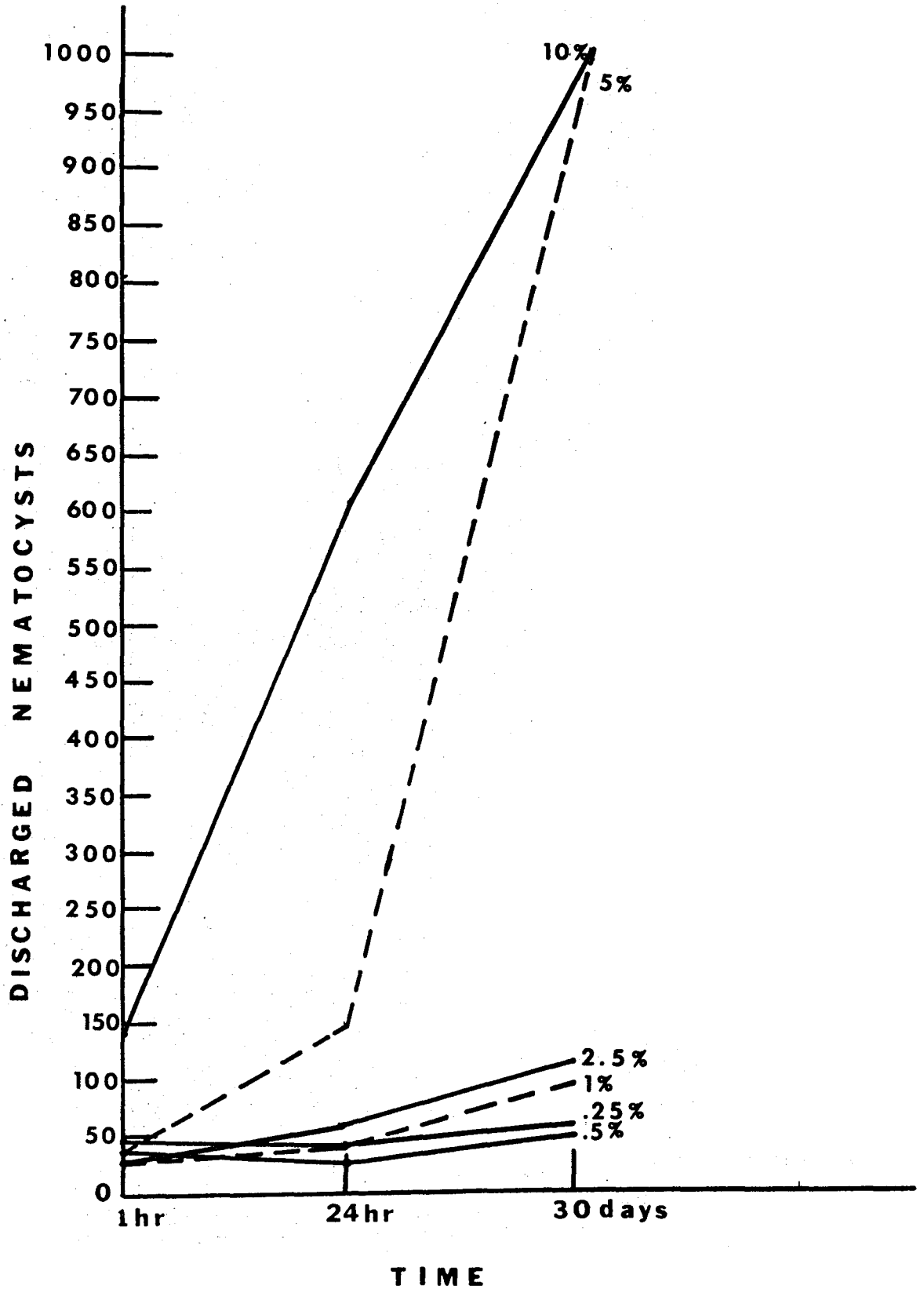
1000
950
900
850
800
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
DISCHARGED NEMATOCYSTS
10%
5%
2.5%
1%
.25%
.5%
1hr
24hr
30 days
TIME

TABLE 19. - Analysis of variance for discharge data for nematocysts in suspension treated with sodium hydroxide

Analysis of Variance			
Source of Variation	d.f.	MS	F
Between subjects	23	142992.33	
concentration (C)	5	647156.29	219.20 *
error (C)	18	2952.34	
Within subjects	48	79830.24	
time (T)	2	680517.54	229.31 *
C x T	10	236398.16	79.65 *
error (T)	36	2967.63	

$F_{.95}(5,18) = 2.77$

$F_{.95}(2,36) = 3.28$

$F_{.95}(10,36) = 2.12$

TABLE 20. - Analysis of variance of discharge data for nematocysts in suspension treated with sodium hydroxide

Analysis of Variance			
Source	d.f.	MS	F
Time at .25% (C)	2	284.25	.10
Time at .5% (C)	2	106.09	.04
Time at 1% (C)	2	4320.75	1.45
Time at 2.5% (C)	2	5489.09	1.84
Time at 5% (C)	2	1112377.59	374.83 *
Time at 10% (C)	2	739840.6	249.33 *
error	36	2967.63	

$F_{.95\ (2,36)} = 3.28$

TABLE 21. - Means of discharge data for nematocysts in suspension treated with ammonium chloride

AMMONIUM CHLORIDE		TABLE OF MEANS				
Concentration (C)	pH	Time periods (T)				
		1hr	24hrs	30 days	60 days	total
.25%	6.7	43.3	89.0	37.0	39.5	52.2
.5%	6.6	39.0	68.5	69.8	49.0	56.5
1%	6.5	54.0	64.0	36.3	49.3	50.0
2.5%	6.3	72.0	40.5	60.8	46.0	54.8
5%	6.2	48.3	44.3	36.8	39.5	42.1
10%	6.0	56.5	56.8	25.3	34.5	48.3
total		52.2	60.5	44.3	46.3	50.8

FIGURE 5. - Graph of means for nematocysts in suspension treated with ammonium chloride

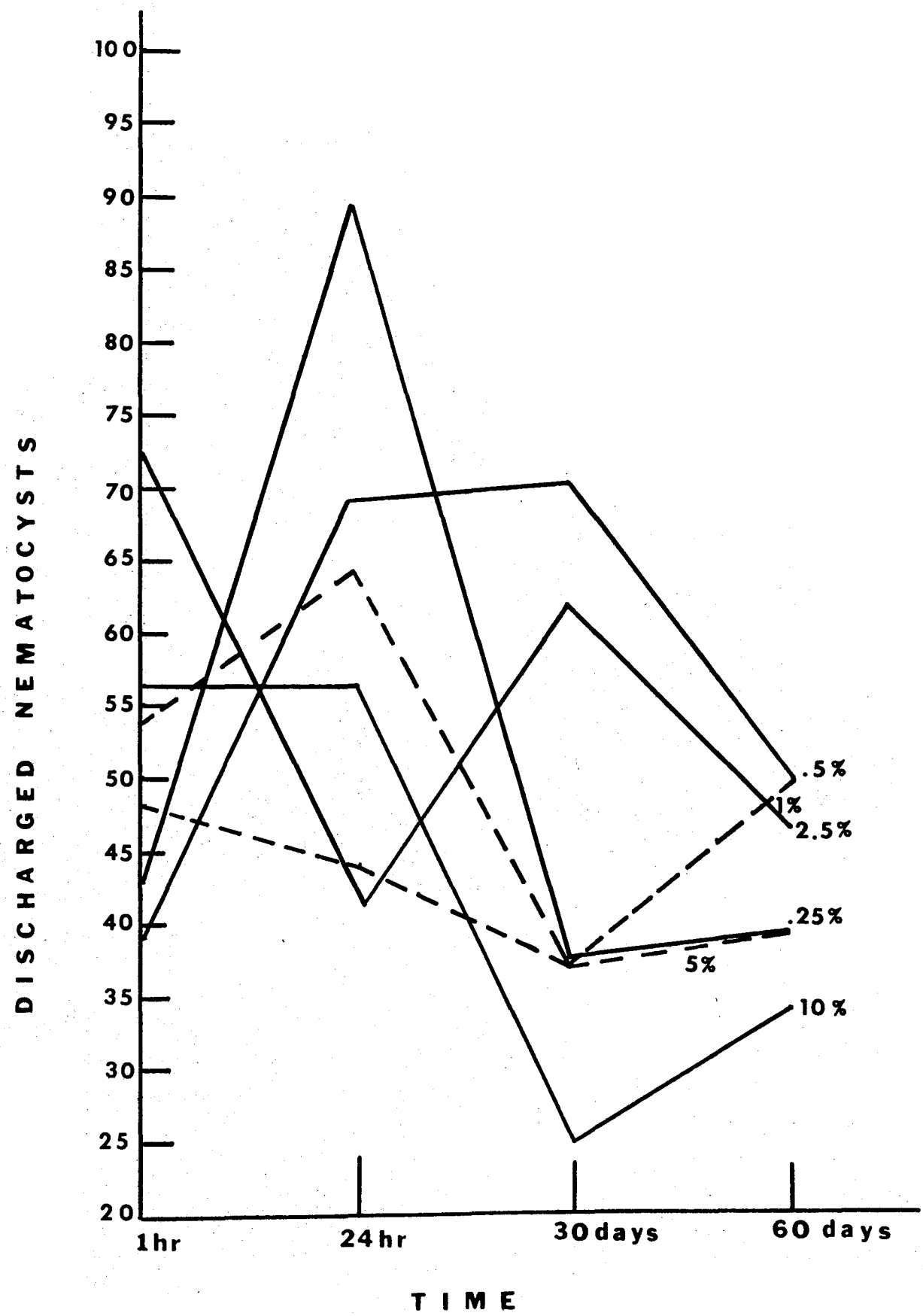
DISCHARGED NEMATOCYSTS
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
1hr
24hr
30days
60 days
TIME
.5%
1%
2.5%
.25%
5%
10%

TABLE 22. - Analysis of variance for discharge data for nematocysts in suspension treated with ammonium chloride

Analysis of Variance			
Source of Variation	d.f.	MS	F
Between subjects	23	1251.41	
concentration (C)	5	419.37	.28
error (C)	18	1482.41	
Within subjects	72	771.73	
time (T)	3	1273.48	1.79
C x T	15	891.99	1.25
error (T)	54	710.45	

$F_{.95\ (5,18)} = 2.77$

$F_{.95\ (3,54)} = 2.79$

$F_{.95\ (15,54)} = 1.87$

TABLE 23. - Means of discharge data for nematocysts in suspension treated with food extracts

FOOD EXTRACTS		TABLE OF MEANS			
Concentration (C)	pH	Time periods (T)			
		1hr	24hrs	30 days	total
Saliva	8.4	48.5	39.8	34.3	40.8
Beef	7.2	50.8	49.8	29.8	43.4
Fish	7.7	59.0	31.3	75.8	55.3
Crab	7.9	51.5	48.0	47.3	48.9
	total	52.4	42.2	46.8	47.1

FIGURE 6. - Graph of means for nematocysts in suspension treated with food extracts

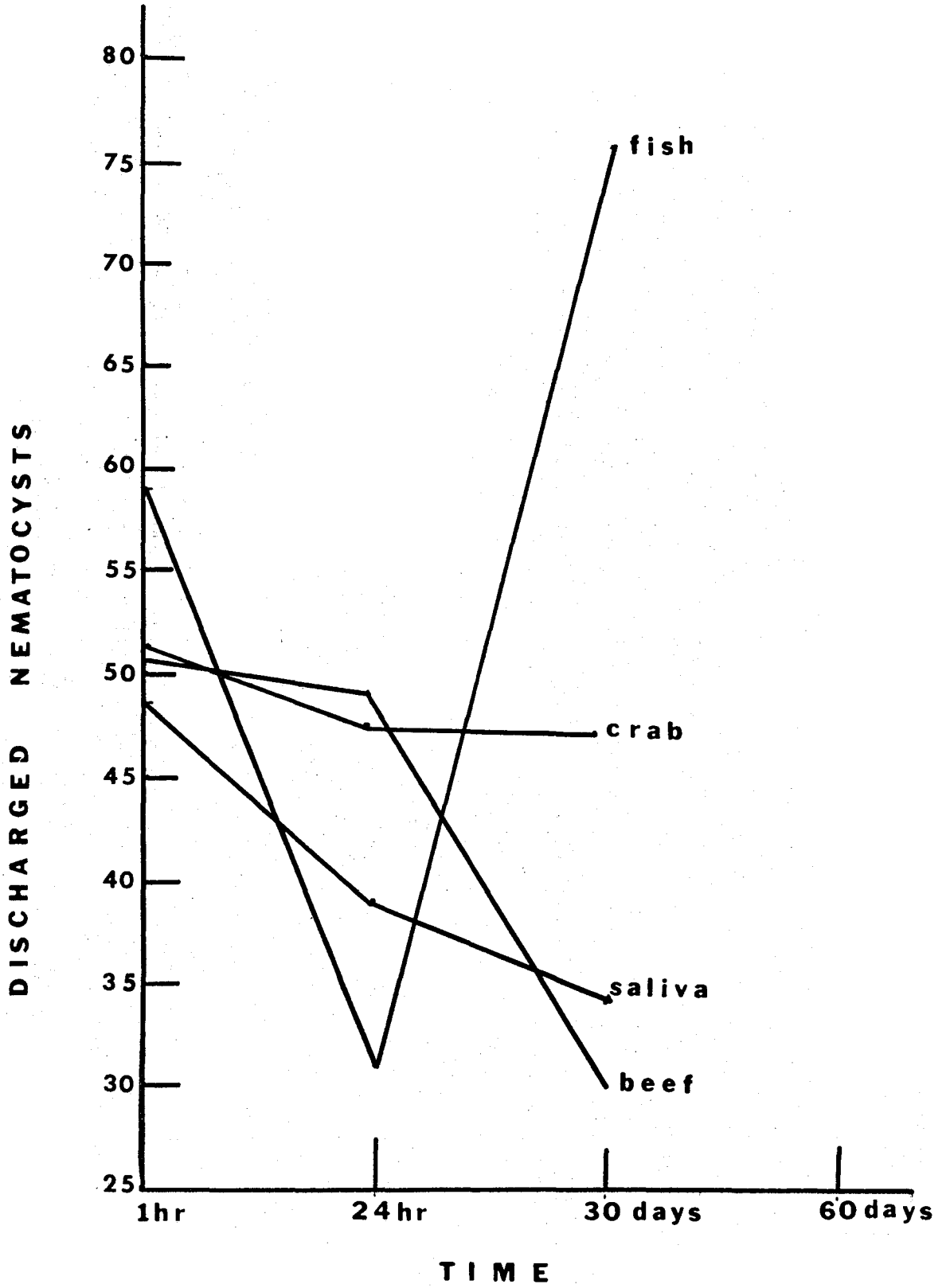
80
75
70
65
60
55
50
45
40
35
30
25
DISCHARGED NEMATOCYSTS
fish
crab
saliva
beef
1hr
24hr
30 days
60 days
TIME

TABLE 24. - Analysis of variance for discharge data for nematocysts in suspension treated with food extracts

Analysis of Variance			
Source of Variation	d.f	MS	F
Between subjects	15	1025.95	
concentration (C)	3	495.69	.33
error (C)	12	1158.51	
Within subjects	32	869.25	
time (T)	2	421.94	.46
C x T	6	795.72	.86
error (T)	24	924.91	

$F_{.95}(3,12) = 3.49$

$F_{.95}(2,24) = 3.40$

$F_{.95}(6,24) = 2.51$

TABLE 25. - Newman-Keuls test of ordered means - Means of concentration for nematocysts in suspension treated with acetic acid

			Concentration (C)			
			2.5%	50%	10%	5%
(C)	Mean	Control Mean 75,00	93.81	105.06	107.38	107.56
	75.00	---	18.81	30.06*	32.38*	32.56*
2.5%	93.81		---			13.75
50%	105.06			---		2.50
10%	107.38				---	
5%	107.56					---

TABLE 26. - Newman-Keuls test of ordered means - Mean of time for nematocysts in suspension treated with acetic acid

			Time (T)			
			1hr	60 days	24hrs	30 days
(T)	Mean	Control Mean 75.00	95.81	101.56	103.19	113.25
	75.00	---	20.81*	26.56*	28.19*	38.25*
1hr	95.81		---		7.38	17.44*
60 days	101.56			---		11.69
24 hrs	103.19				---	10.06
30 days	113.25					---

TABLE 27. - Newman-Keuls test of ordered means - Means of concentration for nematocysts in suspension treated with hydrochloric acid

			Concentration (C)			
			2.5%	5%	50%	10%
(C)	Mean	Control Mean 75.00	96.1	103.6	105.1	106.1
	75.00	---	21.1*	28.6*	30.1*	31.1*
2.5%	96.1		---			10.0
5%	103.6			---		2.5
50%	105.1				---	1.0
10%	106.1					---

TABLE 28. - Newman-Keuls test of ordered means - Means of time for nematocysts in suspension treated with hydrochloric acid

			Time (T)		
			1hr	24hrs	30 days
(T)	Mean	Control Mean 75.00	98.3	103.8	106.0
	75.00	---	23.3*	28.8*	31.0*
1hr	98.3		---		7.7
24hrs	103.8			---	2.2
30 days	106.0				---

TABLE 29. - Newman-Keuls test of ordered means - Means of concentration for nematocysts in suspension treated with salt solutions

		Concentrations (C)							
		17.5%	12.5%	Control	2.5%	5%	1%	.5%	Dist. H_2O
(C)	Mean	64.8	72.8	75.00	77.8	86.3	91.0	92.8	95.8
17.5%	64.8	---	8.0	10.2	13.0	21.5	26.2	28.0	31.0
12.5%	72.8		---						23.0
	75.00			---					20.8
2.5%	77.8				---				18.0
5%	86.3					---			9.5
1%	91.0						---		4.8
.5%	92.8							---	3.0
Dist. H_2O	95.8								---

TABLE 30. - Newman-Keuls test of ordered means - Means of time for nematocysts in suspension treated with salt solutions

			Time (T)			
			60 days	24hrs	30 days	1hr
(T)	Mean	Control Mean 75.00	79.1	81.5	82.9	88.4
	75.00	---	4.1	6.5	7.9	13.4
60 days	79.1		---			9.3
24 hrs	81.5			---		6.9
30 days	82.9				---	5.5
1 hr	88.4					---

TABLE 31. - Newman-Keuls test of ordered means - Means of concentration for nematocysts in suspension treated with sodium hydroxide

		Concentration (C)						
		.5%	Control	.25%	1%	2.5%	5%	10%
(C)	Mean	37.66	40.0	50.25	58.0	68.08	394.09	581.66
.5%	37.66	---	2.34	12.59	20.3	30.42	356.43*	544.00*
	40.0		---			24.08	354.09*	541.66*
.25%	50.25			---		17.83	343.84*	531.41*
1%	58.0				---	10.08	336.09*	523.66*
2.5%	68.08					---	326.01*	513.58*
5%	394.09						---	87.57*
10%	581.66							---

TABLE 32. - Newman-Keuls test of ordered means - Means of time for nematocysts in suspension treated with sodium hydroxide

			Time (T)		
			1hr	24hrs	30 days
(T)	Mean	Control Mean 40.0	55.83	154.91	384.12
	40.0	---	15.83	114.91 *	344.12 *
1 hr	55.83		---	99.08 *	328.29 *
24 hrs	154.91			---	229.21 *
30 days	384.12				---

TABLE 33. - Newman-Keuls test of ordered means - Means of concentration for nematocysts in suspension treated with ammonium chloride

			Concentration (C)					
			5%	10%	1%	.25%	2.5%	.5%
(C)	Mean	Control Mean 40.0	42.1	48.3	50.9	52.2	54.8	56.5
	40.0	---	2.1	8.3	10.9	12.2	14.8	16.5
5%	42.1		---					14.4
10%	48.3			---				8.2
1%	50.9				---			5.6
.25%	52.2					---		4.3
2.5%	54.8						---	1.7
.5%	56.5							---

TABLE 34. - Newman-Keuls test of ordered means - Means of time for nematocysts in suspension treated with ammonium chloride

			Time (T)			
			30 days	60 days	1hr	24hrs
(T)	Mean	Control Mean 40.0	44.3	46.3	52.2	60.5
	40.0	---	4.3	6.3	12.2	20.5
30 days	44.3		---			16.2
60 days	46.3			---		14.2
1hr	52.2				---	8.3
24hrs	60.5					---

TABLE 35. - Newman-Keuls test of ordered means - Means of food extracts for nematocysts in suspension

			Food Extract			
			Saliva	Beef	Crab	Fish
Food Extract	Mean	Control Mean 40.0	40.8	43.4	48.9	55.3
	40.0	---	.8	3.4	8.9	15.3
Saliva	40.8		---			14.5
Beef	43.4			---		11.9
Crab	48.9				---	6.4
Fish	55.3					---

TABLE 36. - Newman-Keuls test of ordered means - Means of time for nematocysts in suspension treated with food extracts

			Time (T)		
			24hrs	30 days	1hr
(T)	Mean	Control Mean 40.0	42.2	46.8	52.4
	40.0	---	2.2	6.8	12.4
24hrs	42.2		---		10.2
30 days	46.8			---	5.6
1hr	52.4				---

VITA

Christa Merz Hubbard was born in Uttrichshausen, Germany on January 1. 1945. In October, 1951 she immigrated with her parents to the United States and settled in Richmond, Virginia. She attended public and parochial schools in the Richmond area and was graduated from Highland Springs High School in June, 1963.

She entered Westhampton College of the University of Richmond in September, 1963 and received the Bachelor of Arts degree in biology and German in June, 1967. At Westhampton she served as a College Government Representative and Treasurer of the Senior Class, and was elected to Beta Beta Beta, honorary biological fraternity.

After graduation from Westhampton she taught seventh grade science at Fairfield Junior High School in Henrico County, Virginia for two years.

In September, 1969 she returned to the University of Richmond and began graduate work in biology. She completed requirements for the Master of Arts degree in biology in August, 1974.

In September, 1971 she again returned to Fairfield Middle School where she is presently teaching eighth grade earth science and is chairman of the Science Department. She is married to Vincent Alden Hubbard.