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On the nature and action of coelenterate toxins*

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Summary—Tetramethylammonium, known to occur in sea anemones, has been thought to be one of constituents of coelenterate nematocyst toxins. To test this suggestion it was first shown that the tendency to drop legs, or autotomize, in the fiddler crab *Uca mordax*, was reduced by previous injection of extracts of tentacles of two species of anemones and of *Physalia*, the Portuguese-man-of-war. It was then found that injected tetramethylammonium chloride or acetylcholine had similar actions to those of the extracts.

Further experiments of a comparable nature were done at another location, using as a test animal the shore crab, *Hemigrapsus nudus*. Extracts of tentacles of *Metridium*, when injected into *H. nudus*, produced spontaneous autotomy of walking legs and chelae, followed by a degree of paralysis dependent upon the dose.

After the injection of tetramethylammonium chloride into *H. nudus* there were no spontaneous autotomies, but there was a type of paralysis similar to that produced by tentacle extracts.

Tetraethylammonium chloride, and a related compound called Banthine, were highly effective in blocking the autotomy-inducing and paralyzing actions of *Metridium* tentacle extracts. This is added evidence that a quaternary ammonium base similar to tetramethylammonium occurs in coelenterate tentacle extracts.

When *Metridium* and *Cyanea* tentacle extracts were tested on an isolated mollusc heart, evidence for the presence of an excitor amine was obtained. It appeared not to be histamine. Later experiments strongly suggested that the material with the marked action on the molluscan heart was 5-hydroxytryptamine, a known histamine-releaser and potent pain producer.

Through the use of paper chromatography further evidence has been obtained for the presence of 5-hydroxytryptamine in extracts of tentacles of *Metridium* and *Physalia*. This approach has also shown the presence of two or more quaternary ammonium bases, one of which has been tentatively identified as urocanylcholine, also known as murexine.

The pain producing factor in coelenterate tentacle extracts is probably 5-hydroxytryptamine; while paralysis could be due to two or more related quaternary ammonium bases such as tetramethylammonium and urocanylcholine.

The painful and paralyzing nature of the "sting" of certain jellyfishes and the siphonophore, *Physalia*, is well known. Less well known is the fact that all coelenterates possess stinging organelles, called nematocysts, which serve two main functions; one, defence, and two, immobilization of prey for ease in feeding. The latter is well illustrated by a hydra subduing and engulfing a large and active Daphnia. The ability to produce a painful sting is not an indication of the amount of toxic substance present in the tentacles of a given species; rather, it is the ability of the nematocysts to penetrate the skin. This ability varies greatly with species, and effective penetration of human skin is restricted to the nematocysts of relatively few species of coelenterates. For example, the sea anemone, *Anemonia sulcata*, has penetrants that enter the skin of the finger sufficiently to cause a stickiness between the tentacles and the skin, but seldom is there subsequent pain. However, when a tentacle of this species is placed on the tongue, a painful and long-lasting burning sensation results, indicating that

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the nematocysts have been able to penetrate the more delicate epithelium of the tongue.

Numerous attempts have been made to determine the nature of nematocyst toxins. Among the earlier studies were those of RICHET, which began in 1902 (for complete report see RICHET and PORTIER (1936)). He isolated two chemically unidentified substances which were named " thalassin " and " congestin ". Two papers summarizing much of the earlier work on nematocyst toxins are those of THIEL (1935) and SONDERHOFF (1936). Recently, BOISSEAU (1952) has used histochemical procedures in an attempt to identify the capsular contents of nematocysts.

Coelenterates feed to a considerable extent on crustaceans, and there are close commensal associations between coelenterates and crustaceans (e.g. the several hermit crab–sea anemone pairs). It is natural, therefore, that attention should have been paid to the physiological action of coelenterate extracts when injected into crustaceans. Many interesting observations of this sort have been made by CANTACUZÈNE (1925 A, B, 1926), CANTACUZÈNE and COSMOVICI (1925), CANTACUZÈNE and DAMBOVICEANU (1934 A, B, C), COSMOVICI (1925 A, B, C, D, E), REY (1940), MANUTA (1943) and others. It is clear that extracts of coelenterate tentacles (the chief nematocyst-bearing structures) have a powerful paralyzing action on crustaceans; also, that a crustacean which lives in close association with a coelenterate develops a resistance to the paralyzing factor(s).

This paper will summarize a series of observations on the chemical nature and physiological actions of nematocyst toxins. The studies were begun in 1950 and were subsequently carried on as part of a larger study on invertebrate pharmacology. Various localities have provided abundant experimental material, and different portions of the work have been carried out at the Lerner Marine Laboratory, Bimini, British West Indies; the Marine Field Laboratories of the University of Washington, Friday Harbor, Washington; the Plymouth Laboratory, Plymouth, England; and the Bermuda Biological Station. I am indebted to the Directors and Staffs of these Marine Stations for the excellent facilities that have been provided. Work toward the identification of the active materials, mainly by use of paper chromatography, has been done at Harvard University. Because the work at each laboratory was carried out on local representatives, it cannot readily be integrated. It seems best therefore, to give a brief account of the findings made at each location.

In view of Professor BIGELOW'S interest in the coelenterates, and especially the scyphomedusae, it is not unfitting that a discussion of nematocyst toxins be included in this volume. It is a privilege to dedicate this paper to one from whom I have learned so much.

I. OBSERVATIONS MADE AT BIMINI (MARCH 27–APRIL 7, 1950)

The earliest observations in this series were made at Bimini, and concerned mainly the effect of tentacle extracts and certain selected chemicals on the autotomy reflex of a species of fiddler crab. WELSH and HASKIN (1939) had found the frequency of dropping of legs in certain crustaceans to be a sensitive indicator of the activity of injected substances, such as acetylcholine and adrenaline. On the assumption that nematocyst substance might act on some step in conduction or transmission of nerve impulses, the autotomy reflex was chosen as an indicator of such action.

Preliminary tests of three species of fiddler crabs found on North Bimini (*Uca* *heterochelos* (Lamarck), *U. leptodactyla* Rathbun and *U. mordax* (Smith))* showed *U. mordax* to be most suitable. In all experiments to be reported here, this species was used.

Tentacle extracts were prepared from *Physalia*, the Portuguese-man-of-war, and from two common sea anemones, *Condylactis gigantea* and *Aiptasia sp.* The extracts were prepared by grinding a known volume of tentacles with an equivalent volume of sea water, either with or without the addition of enough hydrochloric acid to make the suspension weakly acid to litmus paper. If necessary, after filtration the pH was adjusted with $NaHCO_3$ until the solution was neutral. Extracts were diluted as indicated, and injected at the base of one of the last pair of walking legs in amounts varying from 0·02 ml to 0·05 ml depending on the size of the crab. Crabs were injected in lots of five, and after 5–10 minutes the walking legs were grasped firmly with forceps for one or two seconds while the crab was held in a fixed position. This was usually a sufficient stimulus to produce autotomy in an untreated crab. Legs were grasped in a random order (cf. WELSH and HASKIN, 1939) and a record made of the number of autotomies in each crab. For each test the number of legs autotomized appears as the numerator of a fraction with a constant denominator of 40, this being the total number of walking legs possessed by 5 crabs.

Uninjected *U. mordax*, stimulated in the manner indicated, autotomized nearly all of their walking legs. Two lots of 5 males each dropped 36 of 40 legs. One lot of females dropped 37 of 40 legs. Two lots of males injected with 0·05 ml of sea water dropped 37 of 40 and 39 of 40 legs.

Table I shows the effects of extracts of three different species on the tendency to autotomize. It will be seen that the higher concentrations reduced the tendency to autotomize and even prevented any significant number of autotomies at the highest concentration used. The presence of a paralyzing agent in the extracts would account for such results.

Table I

Effects of several dilutions of tentacle extracts on the autotomy reflex in Uca mordax

Species	*No dilution*	1:10	1:100	1:1000	1:10,000
Physalia	0/40	5/40	23/40	40/40	
Aiptasia	0/40	7/40	40/40		
Condylactis		0/40	9/40	14/40	36/40

The reason for the great effectiveness of the extract of tentacles of the sea anemone, *Condylactis*, is not entirely clear. This anemone may actually carry a larger amount of paralyzing substance than *Physalia*, in a given weight of tentacle, but the ground tentacles used in making this extract stood for a longer period before filtering than in other extractions. This may have released more active material into solution.

Since *U. mordax* had responded to the extracts by showing clear signs of paralysis and by a reduced tendency to autotomize their appendages, they were obviously

* Kindly identified by Dr. FENNER A. CHACE of the National Museum, Washington, D.C.

suitable for further study of possible paralyzing agents. The first to be tried was tetramethylammonium chloride (Me_4N) for the following reasons: (1) it is a normal constituent of coelenterates (ACKERMAN, HOLTZ and REINWEIN, 1923, and the hydroxide was named tetramine by these workers); (2) it has long been known to have curare-like properties in the vertebrates; and (3) there were earlier indications that it caused paralysis in insects and crustaceans (WELSH, unpublished).

Tetramethylammonium chloride was dissolved in sea water and appropriate dilutions were made such that various amounts could be introduced by injection of a constant volume of fluid. After injection, legs were tested in the manner described for extracts. The results of two series of tests are given in Table II. It is clear that Me_4N is a moderately effective inhibitor of autotomy in *U. mordax*.

Since a tetraethylammonium salt (Et_4N) often has an action opposing that of Me_4N (e.g. WELSH and TAUB, 1950), this was injected in amounts of 0·5 mg and 0·05 mg. At the lower concentration the frequency of autotomy was 36/40, 39/40 and 40/40 in three experiments. After Et_4N, legs autotomized with remarkable ease.

Table II

Effect of several concentrations of tetramethylammonium chloride (*Me_4N*) *on the autotomy reflex of* Uca mordax

Me_4N	5 *mg*	0·5 *mg*	0·05 *mg*	0·005 *mg*
Series 1	0/40	11/40	30/40	38/40
Series 2	0/40	22/40	30/40	40/40

If acetylcholine is involved in the normal autotomy reflex in *U. mordax*, as it appears to be in *Petrolisthes* (WELSH and HASKIN, 1939), injected acetylcholine might facilitate autotomy or it might reduce it by raising the level to the inhibitory or paralytic range. With *U. mordax*, injected acetylcholine (0·5 mg/crab) reduced the frequency of autotomy. The triethyl analogue of acetylcholine (Et_3Ach) may antagonize acetylcholine (WELSH and TAUB, 1950). When 0·5 mg. of Et_3Ach was injected into *U. mordax*, legs autotomized with remarkable ease when grasped, and in each series there was 100 per cent autotomy. When Me_4N and Et_4N, or Ach and Et_3Ach, were injected simultaneously in 0·5 mg amounts, there was a significant antagonistic action between the pairs of compounds, and the tendency to drop legs was greater than with Me_4N or Ach injected alone.

Atropine, which blocks Ach at certain neuro-effector junctions, facilitated autotomy in *U. mordax*, when injected in a dose of 0·005 mg. Eserine, which prevents Ach from destruction by cholinesterase and therefore permits its accumulation, reduced autotomies to 31 of 40 at 0·001 mg and to 0 of 40 at 0·005 mg.

These results suggest that acetylcholine is involved in the autotomy reflex in *U. mordax*, and that any increase over normal body concentration results in some degree of paralysis. Me_4N, frequently shown to act like Ach in small amounts and to be paralytic in larger amounts, produces paralysis resembling that produced by the coelenterate extracts. Agents which may antagonize Ach and Me_4N, such as Et_3Ach and Et_4N, have actions on *U. mordax* which support the view that a methylated quaternary ammonium base might be a constituent of nematocyst toxins. There is yet no proof, however, that Me_4N ("tetramine"), although found in large amounts in coelenterates, is the paralyzing agent in nematocysts.

A further observation made in Bimini deserves to be reported. The sea anemone, *Aiptasia*, was often found in the Bimini lagoon, occupying an empty conch shell. Such shells frequently contained a single specimen of the red snapping shrimp, *Crangon armatus* (Rathbun).* Hermit crabs living in close association with sea anemones (especially *Eupagurus prideauxi*, which is nearly enclosed by the cloak anemone, *Adamsia palliata*) are known to be highly resistant to nematocyst toxins (CANTACUZÈNE, 1925 B; CANTACUZÈNE and DAMBOVICEANU, 1934 C; REY, 1940; MANUTA, 1943). To test for a possible acquired resistance of *C. armatus* to nematocyst toxins, appropriate doses of anemone tentacle extract were injected into several specimens. Three types of crustaceans were used as controls (*U. mordax*, *Callianassa sp.* and an unidentified shore crab). *Crangon armatus* survived for about 12 hours after injection with an amount of extract which, in less than one hour, killed the other crustaceans not found in association with sea anemones. This provides another example of the acquisition of a resistance to nematocyst toxins by a crustacean living in association with a sea anemone.

II. OBSERVATIONS MADE AT FRIDAY HARBOR (JULY 23, 1951, TO AUGUST 19, 1951)

(a) *Autotomy experiments*

At Friday Harbor, experiments similar to those done at Bimini were performed. As sources of toxins, the tentacles of the sea anemone, *Metridium dianthus*, and the large brown jellyfish, *Cyanea capillata*, were used. Tentacles were cut off and their volume determined. Hydrochloric acid was then added to pH 3–4. The tentacles were homogenized in a Waring blender and the homogenate strained through fine bolting silk. The pH of the liquid obtained was about pH 6. This extract was diluted with various proportions of sea water before use.

As a test animal the common shore crab of that region, *Hemigrapsus nudus*, was used. To compensate in part for a considerable size range, the crabs were sorted into two groups, those weighing about 2 to 4 gm and those weighing about 5 to 8 gm. Extracts and drugs were injected into the smaller crabs in a volume of 0·05 ml, while the volume used for the larger size was double this. Thus the amount of active material administered was, on the average, adjusted to body weight, since each test was done on a group of ten crabs.

Table III

Effect of extract of tentacles of Metridium dianthus *on autotomy in* Hemigrapsus nudus

Dilution	1:10	1:20	1:50	1:100	1:1,000
Total of walking legs autotomized	80/80	78/80	72/80	74/80	78/80
Spontaneous autotomies	42†	28	6	0	0

† A second extract at this dilution when tested on two lots of 10 crabs each gave 38 and 40 spontaneous autotomies.

* Kindly identified by MR. JOHN C. ARMSTRONG.

Preliminary autotomy experiments soon showed that *H. nudus* differed significantly from *U. mordax.* The legs of *Hemigrapsus* usually had to be crushed with heavy forceps to induce autotomy and, after the injection of extracts or drugs, there was no graded response to be seen in the per cent of legs autotomized. However, it was noted that *Metridium* tentacle extract induced spontaneous autotomy of both chelae and walking legs, and the frequency of autotomy was related to dose. The results of a typical experiment are given in Table III. Such a table does not reveal the true nature of the response. In order to do this, the protocol of a typical experiment follows:

July 30, Metridium tentacle extract number 3 (=M-3)

4.25 p.m. Injected 10 *H. nudus* with 0·05 ml M-3, 1:1 with sea water.
4.30 p.m. 17 walking legs and 6 chelae had been spontaneously autotomized. Crabs are paralyzed and fail to right themselves when turned on their backs.
8.00 p.m. All crabs dead.

4.55 p.m. Injected 10 *H. nudus* with 0·05 ml of M-3, diluted 1:10 with sea water.
5.00 p.m. 18 walking legs and 10 chelae autotomized; crabs paralyzed.
8.00 p.m. All dead.

5.08 p.m. Injected 10 *H. nudus* with 0·05 ml of M-3, diluted 1:20 with sea water.
5.15 p.m. 30 walking legs and 11 chelae autotomized; crabs slightly paralyzed.
8.00 p.m. All apparently dead.

5.23 p.m. Injected 10 *H. nudus* with 0·05 ml of M-3, diluted 1:40 with sea water.
5.28 p.m. 21 walking legs and 8 chelae autotomized; crabs appear normal.
8.00 p.m. 3 crabs living but paralyzed.

5.35 p.m. Injected 10 *H. nudus* with 0·05 ml M-3 diluted 1:80 with sea water.
5.40 p.m. 6 walking legs and 2 chelae autotomized; crabs appear normal.
8.00 p.m. 3 crabs living but paralyzed.

5.45 p.m. Injected 10 *H. nudus* with 0·05 ml M-3 diluted 1:150.
5.50 p.m. 3 walking legs and 1 chela autotomized.
8.00 p.m. 9 crabs living but partly paralyzed; few can turn over when placed on backs.

When extracts of *Cyanea* tentacles were injected into *H. nudus*, spontaneous autotomies rarely occurred at any of the dilutions. With dilutions up to 1:50, paralysis occurred, but it was, if anything, less severe than with equivalent amounts of *Metridium* extract.

It will be recalled that tetramethylammonium chloride (Me_4N), like tentacle extracts, facilitated autotomy in *U. mordax.* When Me_4N was injected into *H. nudus*, even in high concentration, it seldom produced spontaneous autotomy, and reduced somewhat the tendency of stimulated legs to autotomize. While its paralyzing action resembled that of extracts of *Cyanea* tentacles, it appeared that *Metridium* extracts contained an active material other than an Me_4N-like component.

At Bimini it had been found that tetraethylammonium chloride (Et_4N) had an action opposite that of Me_4N and, likewise, that the actions of acetylcholine and its triethyl analogue (Et_3Ach) differed one from the other. Large doses of Et_4N could be injected into *H. nudus* with little effect. For example, 5 crabs were injected with 0·05 ml of 10% Et_4N. There were no spontaneous autotomies. In five minutes there were some twitching movements of the legs and the crabs were hyperexcitable, but twenty minutes after the injection they were normal in appearance and action.

If the spontaneous autotomies produced by extracts of *Metridium* tentacles were, in part, due to the presence of an Me_4N-like substance, it might be possible to prevent these autotomies by previous or simultaneous injections of Et_4N. Both procedures were followed. A given extract of *Metridium* tentacles (M-2), when diluted 1:10 with sea water, produced 38 spontaneous autotomies of chelae and walking legs, and produced a moderately severe paralysis. When this extract was diluted 1:10 with 1 % Et_4N, rather than sea water, and 0·05 ml injected, there were *no spontaneous autotomies and no signs of paralysis*. In another experiment a given dilution of extract induced 40 spontaneous autotomies in ten crabs. In a second lot of crabs, 15 minutes after the injection of 0·05 ml of 1 % Et_4N, the same dilution of extract produced no autotomies. Banthine, a drug used clinically to block acetylcholine action in vertebrate autonomic ganglia (as is Et_4N), is a derivative of Et_4N. In one experiment 0·05 ml of 10^{-3} Banthine completely blocked the autotomy-inducing and paralyzing action of a *Metridium* extract (M-3, 1:20) that had produced 50 spontaneous autotomies in a control group of 10 crabs.

These experiments done at Friday Harbor provided further evidence for the occurrence of a quaternary ammonium base (Me_4N derivative) in extracts of coelenterate tentacles. But Me_4N alone could not account for all of the observed actions of the extracts on the autotomy reflex of *Uca mordax* and *Hemigrapsus nudus*.

(b) *Evidence for an amine in tentacle extracts*

It is a common observation that the sting of *Physalia* and certain jellyfishes is followed by a painful burning sensation, localized swelling or wheals, redness and urticarial eruptions. Among the more recent descriptions of these and other symptoms, the following may be referred to for further details: ALLNUTT, 1926; WADE, 1928; FRACHTMAN and McCOLLUM, 1945; McNEILL, 1945; BERNSTEIN, 1947. These symptoms are among those that might be expected if histamine were a constituent of nematocyst toxins or if a histamine releaser were present. Accordingly, crabs were injected with histamine. A dose of 0·05 ml of 1 % histamine caused marked paralysis but no spontaneous autotomies. One-tenth this dose caused a mild spastic stiffening of the legs. Pyribenzamine, an antihistamine, was tested. A dose of 0·05 ml of 1 % pyribenzamine caused rapid autotomy of 70 % of the chelae but no walking legs were dropped, nor could they be made to autotomize by crushing. One-tenth this dose of pyribenzamine clearly inhibited autotomy of legs and antagonized the autotomizing effect of *Metridium* tentacle extract. These results further suggest that a histamine-like substance or a histamine releaser may be a constituent of nematocyst toxins.

(c) *Action of extracts on the mollusc heart*

The isolated ventricle of the heart of the quahog, *Venus mercenaria*, is a sensitive test object for the bioassay of a variety of quaternary ammonium compounds (WELSH and TAUB, 1948; 1950; 1951). Me_4N and many of its derivatives were known to decrease the amplitude and frequency of heart beat. Some amines, such as adrenaline and tyramine, were known to be excitatory when applied to the isolated *Venus* heart. It was decided to test the action of coelenterate tentacle extracts on an isolated mollusc heart.

Exploratory tests of hearts of several molluscs of the Friday Harbor region indicated that the heart of the horse clam, *Schizothaerus nuttallii*, was highly suitable for determining the action of crude extracts of *Metridium* and *Cyanea* tentacle extracts. Using the isolated ventricle of *Schizothaerus*, as we had previously used the *Venus* ventricle, it was apparent that whole sea-water extracts of tentacles of both *Metridium* and *Cyanea* contained a mixture of heart excitor and inhibitor substances. However, a powerful excitor material usually was dominant and tended to obscure the action of the inhibitor material. The results will not be reported in detail, since later experiments done elsewhere are more readily interpreted. However, a sample set of records, comparing the action of extracts with histamine, deserve to be shown. Fig. 1 shows the marked excitor actions of *Metridium* and *Cyanea* extracts on the *Schizothaerus* heart. The former extract caused an increase in amplitude and frequency and a tonic shortening of the heart (= rise in base line), while the *Cyanea* extract caused an increase in frequency and more marked tonic shortening of the heart. Such differences could be due to relatively greater amounts of the excitor substance in *Cyanea* tentacles.

When histamine was tested on the same heart, it was found to have a weak excitor action when added in an amount to give 0·5 mg histamine dihydrochloride per millilitre of fluid bathing the heart. It is most unlikely that histamine could be the constituent of tentacle extract producing the marked excitation, and we shall see from later experiments that, in fact, it is not. These experiments done at Friday Harbor did, however, prompt further pursuit of the identity of the material with the marked excitor action, and this work will now be reported.

III. OBSERVATIONS MADE AT THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY AND THE LABORATORY, PLYMOUTH, ENGLAND

(a) *Evidence for the presence of* 5-*hydroxytryptamine in tentacle extracts*

In the course of a study of cardio-excitor substances acting on the *Venus* heart, a recently-available indole amine, 5-hydroxytryptamine (5-HT), was tested. It was found to be far more active than other common biological amines such as adrenaline, nor-adrenaline, histamine and tyramine (WELSH, 1953 A). By means of paper chromatography and bioassay, 5-HT was found to occur in the nervous systems of several molluscs, including *Venus mercenaria* (WELSH, 1953 B; 1954). The excitor action of extracts of tentacles of *Metridium dianthus* tested on the *Venus* heart, and of the sea anemone, *Calliactis parasitica*, when tested on the *Buccinum* heart (Fig. 2), so closely paralleled that of 5-HT that an attempt to identify the unknown substance by chromatography was suggested. This was done for extracts of *Metridium* and *Physalia* tentacles, and clear evidence for the occurrence of 5-HT in these extracts was obtained. This study will be reported in some detail elsewhere. When equal weights of body wall and tentacles of *Metridium* were extracted, much more 5-HT was found in tentacles than in body wall. This suggests, but does not prove, that the 5-HT is contained in nematocysts, which are far more numerous on the tentacles than they are in the body wall tissues, even though pieces of acontia were doubtless included with the latter.

5-HT produces pain at a low concentration (10^{-8} g/ml) when applied to a blister area on the forearm (ARMSTRONG, DRY, KEELE and MARKHAM, 1953). 5-HT is also a very effective releaser of histamine (FELDBERG and SMITH, 1953). Thus, through direct action or through the release of histamine, 5-HT in nematocyst toxin could be responsible for the itching and burning, as well as the weals and haemorrhagic condition, that may follow contact with certain coelenterates. Recently, JAQUES and SCHACHTER

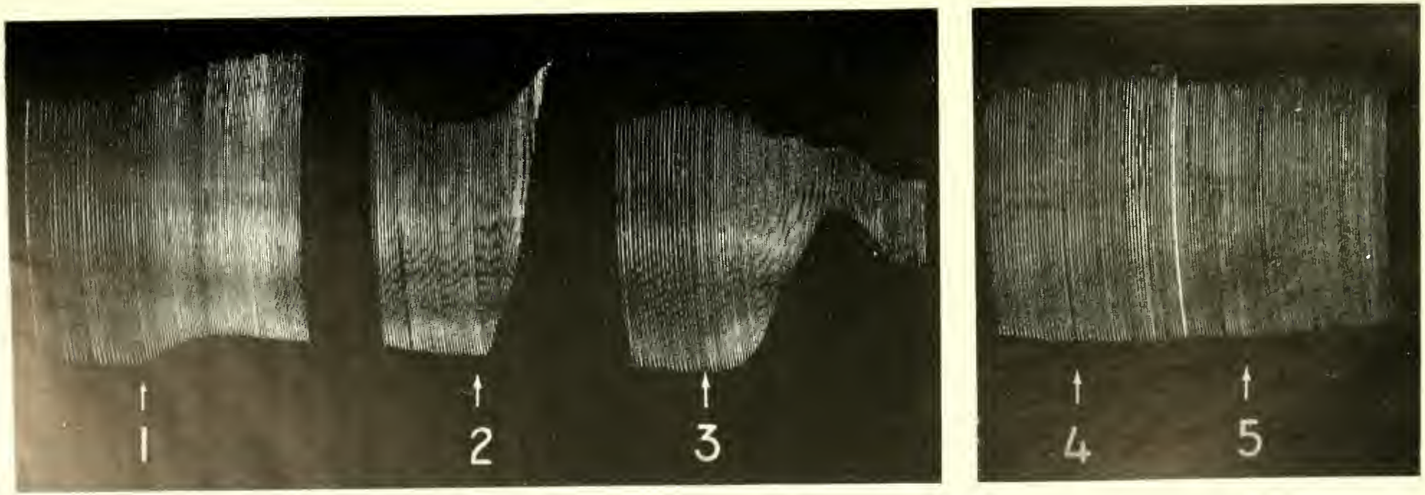

Fig. 1. Action of *Metridium* and *Cyanea* tentacle extracts compared with histamine on the isolated heart of the clam, *Schizothaerus*. Volume of heart bath 20 ml. Substances added to bath at arrows and action recorded for approximately 3 minutes. A 10 minute period of washing between each test.

(1) 1·0 ml undiluted *Metridium* tentacle extract.
(2) 0·5 ml undiluted *Cyanea* tentacle extract.
(3) 0·1 ml same *Cyanea* extract as in (2).
(4) 1·0 ml 0·1% histamine dihydrochloride.
(5) 1·0 ml 1·0% histamine dihydrochloride.

Fig. 2. Effect of extract of *Calliactis parasitica* tentacles compared with 5-hydroxytryptamine on the isolated heart of *Buccinum undatum* (Plymouth, England)

(1) 5×10^{-9} g/ml 5-hydroxytryptamine
(2) 5×10^{-8} g/ml 5-hydroxytryptamine
(3) 5×10^{-7} g/ml 5-hydroxytryptamine
(4) Extract of *Calliactis* tentacles

(1954 A) have reported the presence of a histamine releaser in alcohol extracts of sea anemone tentacles. These same authors (1954 B) find that histamine and 5-HT are components of the venom of the common European wasp, *Vespa vulgaris*.

At the Plymouth Laboratory in the summer of 1953, 5-HT (in the form of the double sulphate with creatinine)* was injected into a series of *Portunus depurator*. The amounts varied by a factor of ten from 0·0001 mg to 0·1 mg 5-HT per crab. All concentrations produced excitation, as seen in constant movements of walking legs and chelae, with twitching of legs and rapid movements of the pincers. The larger the dose, the more severe were the symptoms. However, this was not followed by paralysis, and within one to two hours the crabs appeared normal. While 5-HT may be responsible for some of the activity of coelenterate toxin, it is clearly not the crustacean-paralyzing factor. When coelenterates are feeding on animals other than crustaceans, it is conceivable that 5-HT could act as a relaxing agent, since it has been found to relax certain molluscan muscle (TWAROG, 1954).

Some of our chromatograms have given a spot which reacts as an indole amine but which has an Rf value differing from that of 5-HT. It is possible that more than one indole amine exists in coelenterates, as is the case in the skin of certain amphibians (ERSPAMER, 1954).

(b) *Chromatographic evidence for the presence of quaternary ammonium bases in tentacle extracts*

Methods outlined by WHITTAKER and WIJESUNDERA (1952) and BREGOFF, ROBERTS and DELWICHE (1953) for the chromatography of quaternary ammonium bases were used in an attempt to determine whether or not tetramethylammonium (Me_4N) (or a derivative) was present in extracts of *Metridium* and *Physalia* tentacles. This led to a more extended study than was originally contemplated. The results to date will be briefly summarized.

Various methods were used to separate quaternary ammonium bases from other materials in acetone extracts of *Metridium* and *Physalia* tentacles. These partially purified bases were placed on paper and chromatographed with a variety of solvents. Ultraviolet absorption and chemical reagents were used to locate the spots. All procedures gave evidence of two or more quaternary ammonium bases present in the extracts in relatively very large amounts. Often only one large spot would be obtained, and added substances, such as Ach or Me_4N, would run with the unknowns rather than separately, thus suggesting a complex of related materials with a carrier substance. When certain areas were eluted and assayed on the *Venus* heart, the eluates inhibited the beat as does Me_4N, Ach or other quaternary ammonium base with two or more methyl groups on the quaternary nitrogen. The action of these eluates was antagonized by Et_4N and Mytolon (cf. WELSH and TAUB, 1953).

We have tentatively identified one of the bases in the extracts as murexine or urocanylcholine. This quaternary ammonium base was found in large amounts in the hypobranchial glands of the Mediterranean snail, *Murex*, by ERSPAMER (1948). It was chemically identified by ERSPAMER and BENATI (1953) after synthesis by PASINI, VERCELLONE and ERSPAMER (1952). A generous sample of synthetic urocanylcholine has recently been received from Dr. PASINI. It should make possible certain identification of the material in the extract, which is clearly a quaternary ammonium base with

* Kindly supplied by Dr. R. K. RICHARDS, Abbott Laboratories.

an ultraviolet absorption spectrum very similar to, if not identical with, that of urocanylcholine.

Erspamer and Benati (1953) report that urocanylcholine (murexine) has intense curariform action. A more detailed account of this phase of their work has not yet been seen. However, it appears that a substance such as murexine might account, at least in part, for the paralyzing action of nematocyst poison. Since the hypobranchial glands of *Murex* also contain large amounts of 5-HT (see Erspamer (1954) for references), it could be that a combination of this with urocanylcholine yields a mixture with useful properties in defence and for securing food. It would be strange indeed, should this combination of newly discovered compounds be found in such unrelated structures as the nematocysts of coelenterates and the hypobranchial glands of certain mollusca.

REFERENCES

Ackermann, D., Holtz, F. and Reinwein, H. (1923), Reindarstellung und Konstitutionsermittelung des Tetramins, eines Giftes aus *Aktinia equina*. *Zeits. Biol.*, **79**, 113–120.

Allnutt, E. B. (1926), Effects of a sting by a poisonous coelenterate. *J. Roy. Army Med. Corps*, **46**, 211–213.

Armstrong, D., Dry, R. M. L., Keele, C. A. and Markham, J. W. (1953), Observations on chemical excitants of cutaneous pain in man. *J. Physiol.*, **120**, 326–351.

Bernstein, J. (1947), Beware the Portuguese man-of-war. *Nat. Hist.*, **56**, 136–137.

Boisseau, J. P. (1952), Recherches sur l'histochimie des cnidaires et de leurs nématocystes. *Bull. Soc. Zool. Fr.*, **77**, 151–169.

Bregoff, H. M., Roberts, E. and Delwiche, C. C. (1953), Paper chromatography of quaternary ammonium bases and related compounds. *J. Biol. Chem.*, 205, 565–574.

Cantacuzène, J. (1925 a), Action toxique des poisons d'*Adamsia palliata* sur les crustacés décapodes. *C.R. Soc. Biol., Paris*, **92**, 1131–1133.

Cantacuzène, J. (1925 b), Immunité d'*Eupagurus prideauxi*, vis-a-vis des poisons de l'*Adamsia palliata*. *C.R. Soc. Biol., Paris*, **92**, 1133–1136.

Cantacuzène, J. (1926), Activation des poisons de l'*Adamsia palliata* par la lécithine et pouvoir hémolytique. *C.R. Soc. Biol., Paris*, **95**, 118–120.

Cantacuzène, J. and Cosmovici, N. (1925), Action toxique des poisons d'*Adamsia palliata* sur divers invertébrés marins. *C.R. Soc. Biol., Paris*, **92**, 1464–1466.

Cantacuzène, J. and Damboviceanu, A. (1934 a), Caractères biologiques de l'extrait des acconties d'*Adamsia palliata* après deprotéinisation. *C.R. Soc. Biol., Paris*, **117**, 136–138.

Cantacuzène, J. and Damboviceanu, A. (1934 b), Caractères physicochimiques du poison des acconties d'*Adamsia palliata*. *C.R. Soc. Biol., Paris*, **117**, 138–140.

Cantacuzène, J. and Damboviceanu, A. (1934 c), L'action précipitante spécifique du sérum d'*Eupagurus prideauxi* sur le poison d'*Adamsia palliata*. *C.R. Soc. Biol., Paris*, **117**, 269–271.

Cosmovici, N. L. (1925 a), L'action des poisons d'*Adamsia palliata* sur les muscles de *Carcinus moenas*. *C.R. Soc. Biol., Paris*, **92**, 1230–1232.

Cosmovici, N. L. (1925 b), L'action des poisons d'*Adamsia palliata* sur le coeur de *Carcinus moenas*. *C.R. Soc. Biol., Paris*, **92**, 1300–1302.

Cosmovici, N. L. (1925 c), Les poisons de l'extrait aqueux des tentacules et des nématocystes d'*Adamsia palliata* sont-ils détruits par l'ébullition? Essais d'adsorption. *C.R. Soc. Biol., Paris*, **92**, 1373–1374.

Cosmovici, N. L. (1925 d), Action convulsivante des poisons d'*Adamsia palliata* sur le *Carcinus moenas* *C.R. Soc. Biol., Paris*, **92**, 1466–1469.

Cosmovici, N. L. (1925 e), Autotomie chez *Carcinus moenas*, provoquée par les poisons d'*Adamsia palliata*. *C.R. Soc. Biol., Paris*, **92**, 1469–1470.

Erspamer, V. (1948), Osservazioni preliminari, chimiche e farmacologiche, sulla murexina. *Experimentia*, **4**, 226–228.

Erspamer, V. (1954), Pharmacology of indolealkylamines. *Pharmacol. Revs.*, **6**, 425–487.

Erspamer, V. and Benati, O. (1953), Identification of murexine as β-(imidazolyl-(4))-acryl-choline. *Science*, **117**, 161–162.

Feldberg, W. and Smith, A. N. (1953), Release of histamine by tryptamine and 5-hydroxytryptamine. *Brit. J. Pharmacol.*, **8**, 406–*et seq.*

Frachtman, H. J. and McCollum, W. T. (1945), Portuguese man-of-war stings: a case report. *Amer. J. Trop. Med.*, **25**, 499–500.

Jaques, R. and Schachter, M. (1954 a), A sea anemone extract (thalassine) which liberates histamine and a slow contracting substance. *Brit. J. Pharmacol.*, **9**, 49–52.

Jaques, R. and Schachter, M. (1954 b), The presence of histamine, 5-hydroxytryptamine and a potent slow contracting substance in wasp venom. *Brit. J. Pharmacol.*, **9**, 53–58.

McNeil, F. A. (1945), Injuries by unknown agents to bathers in N. Queensland. *Med J. Australia*, **32** (2), 29.
Manunta, C. (1943), La resistenza dei Paguri al veleno della Attinie. Esperienze su *Paguristes oculatus* Fabr. con *Adamsia rondeletii* Andr. *Thalassia, Ist. Italo-Germanico Biol. Mar., Rovigno d'Istria*, **5**, 1–38.
Pasini, C., Vercellone, A. and Erspamer, V. (1952), Synthese des Murexins (β-(Imidazolyl-4(5))-acryl-cholin *Liebig's Ann.*, **578**, 6–10.
Rey, M. (1940), Effets du poison de différentes actinies sur les crustacés décapodes. *Bull. Soc. Zool. France*, **65**, 58–62.
Richet, C. (1902), Du poison pruritogène et urticant contenus dans les tentacules d'Actinies. *C.R. Soc. Biol., Paris*, **54**, 1438.
Richet, C. (1903), Des poisons contenus dans les tentacules des Actinies, congestine et thalassine. *C.R. Soc. Biol., Paris*, **55**, 246.
Richet, C. and Portier, D. (1936), Recherches sur la toxine des coelentérés et les phénomènes d'anaphylaxie. *Rés. Camp. Sci., Monaco*, **95**, 3–24.
Sonderhoff, R. (1936), Über das Gift der Seeanemonen. I. Ein Beitrag zur Kenntnis der Nesselgifte. *Liebig's Ann.*, **525**, 138–150.
Thiel, M. E. (1935), Über die Wirkung des Nesselgiftes der Quallen auf den Menschen. *Ergeb. u. Fortschr. Zool.*, **8**, 1–35.
Twarog, Betty M. (1954), Responses of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. *J. Cell. Comp. Physiol.*, **44**, 141–164.
Wade, H. W. (1928), Post-mortem findings in acute jelly-fish poisoning with sudden death in status lymphaticus. *Amer. J. Trop. Med.*, **8**, 233–241.
Welsh, J. H. (1953 a), Excitation of the heart of *Venus mercenaria*. *Arch. Exp. Path. u. Pharmakol.*, **219**, 23–29.
Welsh, J. H. (1953 b), The occurrence of an excitor amine in the nervous system of *Buccinum* and its action on the heart. *Anat. Rec.*, **117**, 637.
Welsh, J. H. (1954), Hydroxytryptamine: A neurohormone in the invertebrates. *Federation Proc.*, **13**, 162–163.
Welsh, J. H. and Haskin, H. H. (1939), Chemical mediation in crustaceans. III. Acetylcholine and autotomy in *Petrolisthes armatus* (Gibbes). *Biol. Bull.*, **76**, 405–415.
Welsh, J. H. and Taub, R. (1948), The action of choline and related compounds on the heart of *Venus mercenaria*. *Biol. Bull.*, **95**, 346–350.
Welsh, J. H. and Taub, R. (1950), Structure-activity relationships of acetylcholine and quaternary ammonium ions. *J. Pharmacol. and Exp. Therap.*, **99**, 334–342.
Welsh, J. H. and Taub, R. (1951), The significance of the carbonyl group and ether oxygen in the reaction of acetylcholine with receptor substances. *J. Pharmacol. and Exp. Therap.*, **103**, 62–73.
Welsh, J. H. and Taub, R. (1953), The action of acetylcholine antagonists on the heart of *Venus mercenaria*. *Brit. J. Pharmacol.*, **8**, 327–333.
Whittaker, V. P. and Wijesundera, S. (1952), The separation of esters of choline by filter-paper chromatography. *Biochem. J.*, **51**, 348–351.