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THE DISTRIBUTION OF 5-HYDROXYTRYPTAMINE, TETRAMETHYLAMMONIUM, HOMARINE, AND OTHER SUBSTANCES IN SEA ANEMONES

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Many years ago Richet (1902, 1905) demonstrated the presence of several toxic substances in extracts of tentacles of sea anemones, the study of which incidentally led to the discovery of anaphylaxis. Although several attempts have been made to identify these substances (Richet, 1905; Richet & Portier, 1936; Sonderhoff, 1936), none has been successful. Ackermann, Holtz & Reinwein (1923), however, identified tetramethylammonium in a sea anemone but did not determine its contribution to the toxic properties of anemone extracts.

The present experiments were undertaken in an attempt to identify thalassine, the pruritogenic substance in coelenterates (Richet, 1902, 1905) which is also a potent histamine liberator (Jaques & Schachter, 1954). We soon found, however, that a number of distinct pharmacologically active substances were present in sea anemones, and proceeded to identify them. We have, in fact, identified and determined the distribution of 5-hydroxytryptamine (5-HT), histamine, and tetramethylammonium (TMA); we have also found and studied the properties of another potent substance, probably a protein, which produces a marked and maintained contracture of the frog rectus abdominis muscle and a quick contraction, or twitch, of the sartorius muscle. Thalassine, however, is probably not identical with any of these.

In addition to the above pharmacologically active substances we identified the apparently inactive base, homarine (*N*-methyl picolinic acid betaine), in high concentrations in the tissues of all the sea anemones which we studied, and also in the Portuguese man-of-war, *Physalia*. Homarine, which we found in very high concentrations in some tissues, derived its name from the fact that it was discovered in the tissues of the lobster, *Homarus vulgaris* (Hoppe-Seyler, 1933).

METHODS

Animals. The sea anemones *Calliactis parasitica*, *Actinia equina*, *Anemonia sulcata* and *Metridium senile* were obtained from Plymouth, kept alive in tanks of sea water, and allowed to settle on glass plates. They were removed carefully to avoid closure of the animals and withdrawal of the tentacles. They were quickly frozen either by immersion in liquid nitrogen, or by covering them with powdered dry ice. Animals were dissected into various components—tentacle, outer body wall, and coelenteric structures. The latter unavoidably included some inner surface of the body wall. All tissues were kept frozen throughout dissection, as much frozen sea water as possible was discarded, and the dissected parts were freeze-dried. For the satisfactory separation of the thread-like acontia from the coelenteric tissues, eight specimens of *C. parasitica* were anaesthetized by placing them during the night in an aqueous solution of 7 % magnesium chloride plus one half its volume of sea water.

A single living specimen of *Physalia* (Portuguese man-of-war) was obtained from Hastings, and freeze-dried tissues were prepared as described.

Isolated organ preparations. The isolated rat uterus and guinea-pig ileum were suspended in a 15 ml. bath in Tyrode solution and contractions were recorded by a frontal-writing lever on a smoked drum. The concentration of Ca^{2+} in the Tyrode solution was reduced to half the normal and the bath temperature was kept at 28–30° C (instead of 34° C) with the rat uterus. Atropine, mepyramine and lysergic acid diethylamide were added in concentrations of 10^{-4} g/l. when required. The isolated rectus abdominis muscle of the frog (*Rana temporaria*) was suspended in 8 ml. Ringer's solution, in some instances containing physostigmine (4×10^{-3} g/l.) and contractions were recorded on a smoked drum. Tubocurarine was added to a final concentration of 10^{-3} g/l. when required. The preparation was kept at room temperature. The isolated sartorius muscle of the frog was used in the same way.

The isolated perfused cat skin preparation was prepared as previously described (Feldberg & Paton, 1951; Feldberg & Schachter, 1952).

Extracts. For initial tests freeze-dried tissues were simply macerated in isotonic sodium chloride solution and centrifuged, and the supernatant solution tested. When testing quantitatively for histamine, 5-HT and TMA, the tissues were extracted with saline acidified to pH 3–4 with HCl, heated in a water-bath to 98° C for 1–2 min, cooled, centrifuged, neutralized and tested. In some instances extracts were made with 95 % ethanol, taken to dryness *in vacuo*, redissolved in saline and tested, activity being expressed in reference to the original freeze-dried tissue.

For paper chromatography freeze-dried tissues were first extracted in a small volume of distilled water and centrifuged; the supernatant solution was removed, care being taken to leave the fatty layer behind, and dried *in vacuo*. The residue was then extracted three or four times with small volumes of 95 % ethanol, centrifuged, and the alcohol extract dried. For chromatography, the latter dried residue was taken up in a small volume of water, centrifuged, the supernatant solution reduced in volume *in vacuo*, and applied to the paper.

Paper chromatography. Ascending chromatograms were run during the night at room temperature on Whatman No. 1 paper (except when otherwise stated). The following solvents were used: *n*-butanol–acetic acid–water (Partridge, 1948), *iso*propanol–hydrochloric acid (Lembeck, 1954), *n*-propanol–formic acid (Whittaker & Wijesundera, 1952), and *n*-butanol saturated with 0·2 N ammonium hydroxide. Papers were cut into horizontal strips of 1·0 cm, eluted with saline or Tyrode solution (containing physostigmine, 4×10^{-3} g/l. when required) and tested on isolated muscle preparations. Extracts were prepared as described above and applied as a narrow strip on the paper. The extract applied to a 1 cm strip of paper was derived from 25–50 mg of original freeze-dried tissue.

Chromatograms were viewed under an ultra-violet lamp ('Chromatolete', Hanovia; λ, 254 mμ) and quenching or fluorescent areas marked out. They were developed with one

or more of the following reagents: Pauly's reagent (diazotized sulphanilic acid), ninhydrin–acetic acid (Jepson & Stevens, 1953), ninhydrin, chlorine–starch–iodide (Rydon & Smith, 1952), iodine spray (in 1 % ethanol) or iodine vapour.

Preparation of homarine and other quaternary ammonium compounds

Homarine was prepared as described by Hoppe-Seyler (1933), trimethylammonium iodide by the method of Collie & Schryver (1890), and trimethylpropylammonium iodide by the method of Weiss (1892).

Ultra-violet absorption spectra. The areas on the chromatogram corresponding in R_F value or colour reactions with 5-HT, homarine, etc. were eluted in 2·5 ml. water, and the absorption spectra of the eluate determined in 1·0 cm quartz cells in the Hilger 'Uvispek' against a blank containing an aqueous extract of an equivalent area of the chromatogram corresponding in R_F value to the eluate. Hydrogen ion modifications for spectrophotometry were obtained by addition of small quantities of HCl or NaOH to blank and sample. Concentrations of homarine were calculated by using the molar extinction coefficient quoted by Keyl, Michaelson & Whittaker (1957).

Chemicals and reagents. Histamine and 5-HT were used as acid phosphate and creatinine sulphate, respectively, and values expressed as their bases.

RESULTS

5-*Hydroxytryptamine*

Very high concentrations (up to 600 μg/g freeze-dried tissue) of 5-HT were found in the soft tissues of the coelenteron of the anemone, *C. parasitica*. The concentrations were much less in the column (or body wall) and lowest in the tentacles (Table 1). The exact site of 5-HT in the coelenteric

TABLE 1. Distribution of 5-hydroxytryptamine in the sea anemone *Calliactis parasitica*

Preparation	No. of animals	Test	5-hydroxytryptamine (μg/g freeze-dried tissue) Tentacle	Body wall (column)	Coelenteric tissues
I	5	Rat uterus	10	—	—
II	9	Rat uterus	12	30	—
II	9	Guinea-pig ileum	8	25	—
III	7	Rat uterus	6	36	600
IV	4	Rat uterus	7	15	500

tissues remains undetermined, but it cannot be in the fine thread-like structures present there, the acontia. These structures were pooled from approximately eight specimens of *C. parasitica* and contained only 20 μg/g freeze-dried tissue, whereas the remaining coelenteric tissue contained approximately 500 μg/g. Complete separation of the column from coelenteric tissue is not possible and perhaps the concentration of 5-HT found in the column is exaggerated by contamination with some internal structures.

The identity of the active substance in the extracts with 5-HT was established beyond doubt by a number of pharmacological and chemical

tests. It contracted the isolated guinea-pig ileum (in the presence of atropine and mepyramine) and the rat uterus, and its action on the latter was completely abolished by lysergic acid diethylamide (LSD). Alcohol extracts chromatographed on paper in either *n*-butanol–acetic acid or in *iso*propanol–hydrochloric acid yielded an eluate pharmacologically active when tested on the rat uterus. The area on the paper chromatogram from which this eluate was obtained gave the same colour reactions as 5-HT with ninhydrin, Ehrlich's reagent and Pauly's reagent. It also gave the green ultra-violet fluorescent reaction described by Jepson & Stevens (1953). This area, however, did not correspond exactly with the R_F value of the 5-HT marker strip when relatively impure extracts were chromatographed. Thus, in a typical chromatogram run in butanol–acetic acid, the pharmacologically active eluate had an R_F value of only 0·28, whereas that of a 5-HT marker was 0·40. Similarly, chromatography of the same extract in propanol–formic acid yielded an active eluate with an R_F value of 0·52, whereas that of 5-HT was 0·45 (Fig. 1). In both instances the area on the paper which yielded the pharmacologically active eluate gave the various colour and other reactions for 5-HT which were previously described. After rechromatography in *iso*propanol–hydrochloric acid, however, each of these eluates yielded an active material which now corresponded with synthetic 5-HT in R_F value as well as in the other reactions (Fig. 2). In addition, these purified, rechromatographed eluates yielded ultra-violet absorption curves which closely agreed with those of 5-HT at a pH of 5·6 and also when the curve was determined at pH 12 after addition of 2N-NaOH.

In contrast to the high concentration of 5-HT which we found in *C. parasitica*, we failed to detect it in any of the tissues of *M. senile* or *A. sulcata*. Extracts from these species contained either no 5-HT or certainly less than 2·0 μg/g freeze-dried tissue. We were unable also to detect 5-HT on paper chromatograms of alcohol extracts of these species. The freeze-dried tissues of *Physalia* also contained less than 2·0 μg/g. Extracts of *A. equina* possessed some activity resembling that of 5-HT on the rat uterus, but the low concentrations, approximately 5 μg/g freeze-dried tissue, did not permit a definite identification with 5-HT. Our failure to detect 5-HT in the tissues of *M. senile* or of *Physalia* is in contrast to Welsh's (1956) findings of high concentrations in the tentacles of these animals. Despite the absence of 5-HT, *Physalia* tentacle was extremely toxic. The intraperitoneal injection of freeze-dried tentacle (50 mg/kg) in rats and guinea-pigs caused death within 24 hr with signs of severe pulmonary oedema.

Unheated extracts of *A. equina* and *A. sulcata* contained another substance which also contracted the isolated rat uterus. The delay preceding

contraction in this instance was slightly longer than with 5-HT, the uterus subsequently relaxed more slowly and also tended to become desensitized with repeated tests. The concentration of this substance, expressed as 5-HT equivalent activity, was 20–40 μg/g in *A. equina* and approximately 10 μg/g in *A. sulcata*. It was inactivated by heating to 98° C for 2 min and was non-dialysable.

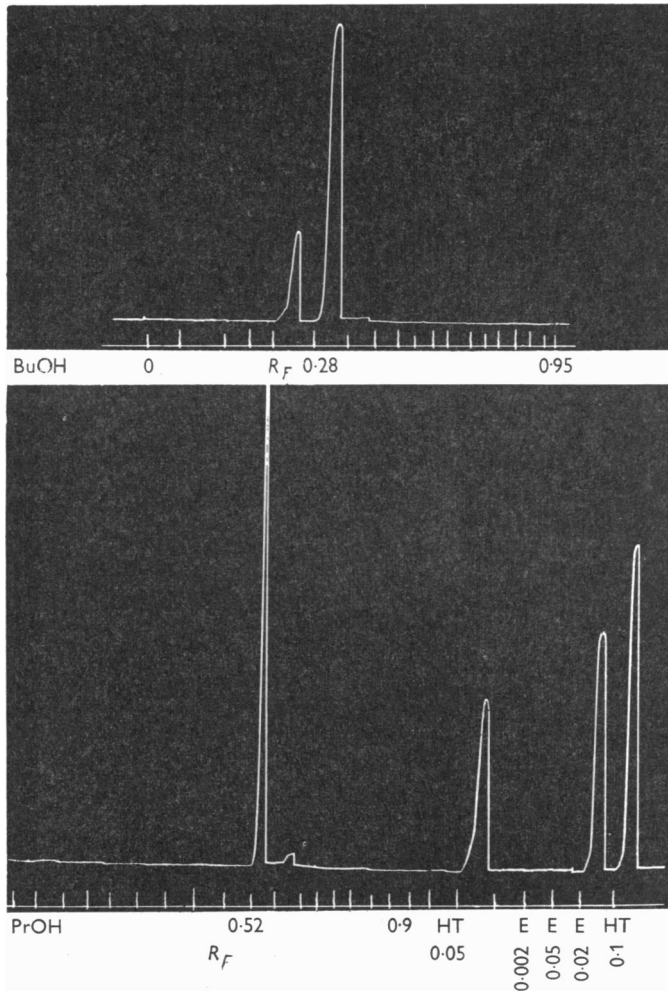


Fig. 1. Contractions of isolated rat uterus (atropine and LSD, 10^{-4} g/l.) to eluates from chromatograms in two different solvents of alcohol extract of the sea anemone, *A. equina*. BuOH, eluates from chromatogram in *n*-butanol–acetic acid solvent. PrOH, eluates from chromatogram in *iso*propanol–hydrochloric acid solvent. HT, 5-HT creatinine sulphate, μg. E, active eluate, ml.

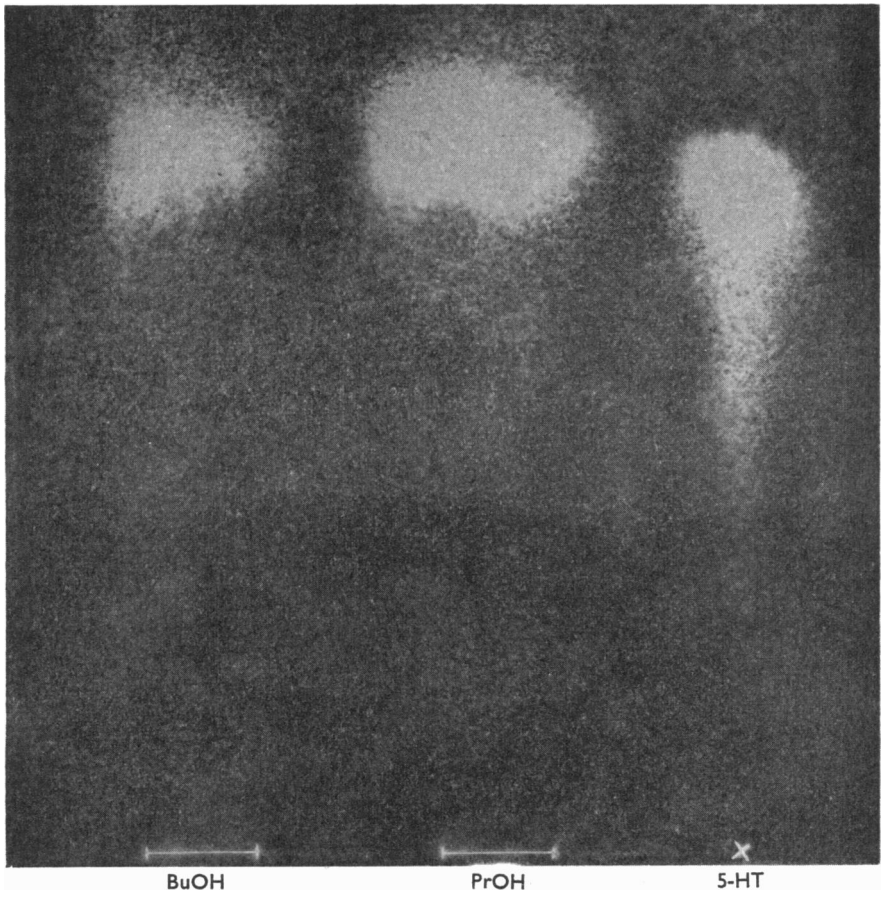


Fig. 2. Rechromatographed extract from *C. parasitica* coelenteric tissue. Ultraviolet light photograph. Solvent, *iso*propanol–hydrochloric acid. BuOH, active eluate from previous chromatogram in *n*-butanol–acetic acid solvent (Fig. 1). PrOH, active eluate from previous chromatogram in *iso*propanol–hydrochloric acid (Fig. 1). 5-HT; marker of 5-HT creatinine sulphate.

Histamine

Histamine was found in the freeze-dried tissues of two of the four species of anemones studied, *A. equina* and *A. sulcata*. It was not detected in the tissues of *C. parasitica*, *M. senile* or in *Physalia* (< 2 μg/g freeze-dried tissue). When histamine was present the highest concentration was in the tentacles (Table 2).

The activity of these extracts was indistinguishable from that of histamine on the guinea-pig ileum, and was antagonized by mepyramine. Furthermore, the extracts of tentacles of *A. equina* chromatographed on

paper in *n*-propanol–formic acid showed a positive Pauly reaction which, on elution, was pharmacologically identical with that of histamine. The high concentrations of 5-HT in the coelenteric tissues of *C. parasitica* made it difficult to eliminate the possible presence of minute amounts of histamine. However, when large amounts of extract of this tissue were tested, the response was completely unaffected by mepyramine, which suggests that histamine was absent.

TABLE 2. Distribution of histamine in various sea anemones

Species	No. of animals	Histamine (μg/g freeze-dried tissue) Tentacle	Body wall (column)	Coelenteric tissues
A. equina I	4	150	*125	—
II	10	50	*35	—
A. sulcata I	2	25	—	—
II	8	20	5	—
III	4	20	8	10
C. parasitica I	5	2	2	2
II	7	2	2	2
M. senile II	4	2	2	2

* In this species a satisfactory separation of body wall (column) from coelenteric tissues was impossible. These values (body wall) are those of the whole animal minus tentacles.

Tetramethylammonium

Saline (0·9 %, w/v) extracts of *A. equina* and *A. sulcata* (but not of *C. parasitica* or *M. senile*) produced a strong contracture of the frog rectus abdominis muscle. Similar extracts of *Physalis* also caused contracture but they were much less effective.

It soon became evident that these extracts of *A. equina* contained two substances, chemically very different, both of which contracted the rectus muscle. One of these was dialysable and soluble in 95 % ethanol, the other non-dialysable and insoluble in ethanol. Thus, following the dialysis of saline extracts of *A. equina* (50–100 mg freeze-dried tentacle in 2 ml. against 8 ml. Ringer's solution for 2 hr) the dialysate produced a contracture of the rectus muscle which resembled that caused by acetylcholine in rate of onset and in subsequent relaxation, whereas the residue produced an irregular and long-maintained contracture. The amount of non-dialysable activity is far greater than that of the dialysable, although the two cannot be compared precisely because of the different types of contracture which they cause.

Alcohol (95 % ethanol) extracts of *A. equina* contained the dialysable substance only. It produced an acetylcholine-like contracture which was completely antagonized by tubocurarine (10^{-3} g/l.) (Fig. 3*a*). It was stable

on heating to 98° C for 5 min in N/100-HCl. Unlike esters of choline it was also stable on heating for 5 min in N/100-NaOH and was unaffected by incubation with horse plasma (1:1 (v/v)) at 37° C for 20 min. It was absorbed by the ion-exchange resin IRC-50 (in the H^+ form) from which it was released by acid. These properties suggested that the alcohol-soluble substance was a simple alkyl quaternary ammonium compound. In subsequent chromatographic experiments on paper it was, in fact, indistinguishable from tetramethylammonium (TMA). The freeze-dried tentacles from different batches of *A. equina* contained 1500–2000 μg/g in terms of TMA equivalent activity in alcohol extracts tested on the frog rectus preparation. The remainder of *A. equina* (minus tentacle) contained approximately one half the concentration of this substance.

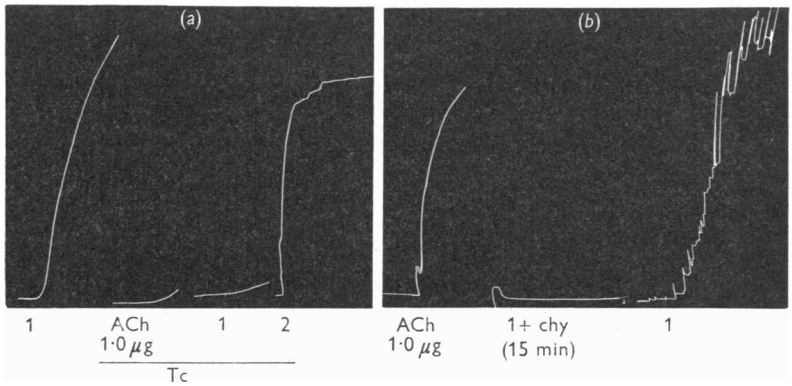


Fig. 3. Response of frog rectus abdominis muscle to extracts of freeze-dried tentacle from sea anemones *A. equina* and *A. sulcata*. ((*a*) and (*b*) are different rectus preparations.) (*a*) Tubocurarine, 10^{-3} g/l. (Tc), abolishes the contracture produced by the alcohol-soluble substance in *A. equina* but effect of saline extract persists. 1, alcohol-soluble material from 50 mg tentacle; 2, saline extract from 2 mg tentacle. (*b*) Saline extract of *A. sulcata* produces contracture with superimposed quick contractions. Effect abolished by incubation of extract with chymotrypsin. 1, saline extract from 5 mg tentacle (0·5 ml.); chy, 50 μg crystalline chymotrypsin (0·5 ml.).

Alcohol extracts of *A. sulcata* had one-tenth or less the activity of *A. equina* in contracting the frog rectus. The low concentrations of this substance in *A. sulcata* did not permit its identification. Alcohol extracts from large amounts of tissue of *C. parasitica*, *M. senile* and *Physalia* failed to cause contracture of the rectus muscle. It is very unlikely, therefore, that these tissues contain significant amounts of pharmacologically active esters of choline or active alkyl quaternary ammonium compounds. These findings differ from those of Welsh (1955), who obtained

evidence of high concentrations of murexine (urocanylcholine) in the tentacles of *Metridium* and *Physalia*.

Alcohol extracts of tentacle or body of *A. equina* chromatographed on paper in *n*-propanol–formic acid yielded an eluate which caused contracture of the frog rectus muscle (Fig. 4), reacted with iodine, and corresponded closely in R_F with TMA iodide. By rechromatographing such an active eluate in two solvents (*n*-propanol–formic acid or *n*-butanol–acetic acid),

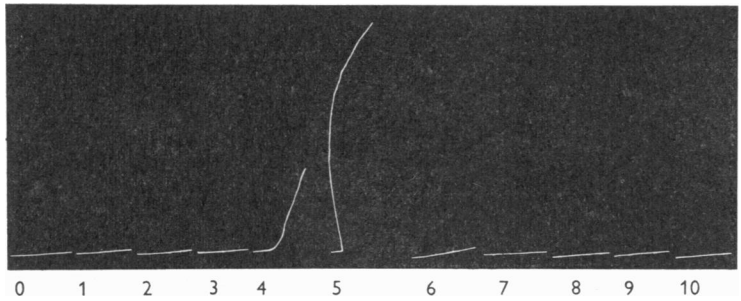


Fig. 4. Contractions of frog rectus abdominis muscle to eluates from paper chromatogram of extract of *A. equina*. 0 = strip 1 cm either side of origin: 2–10, strips 2 cm wide.

TABLE 3. R_F values and other properties of rechromatographed active eluate from extract of *Actinia equina* and various quaternary ammonium compounds in two solvents

Solvents	*n*-propanol–formic acid		*n*-butanol–acetic acid	
Tests	Ultra-violet	I_2 reaction	Ultra-violet	I_2 reaction
TMA	—	0·48 grey	—	0·25 grey
TMEA	—	0·57 orange	—	0·32 orange
TMPA	—	0·70 orange	—	0·40 orange
ACh	—	0·58 orange	—	0·32 orange
Murexine	0·15 *Q*	0·15 orange	0·12 *Q*	0·12 orange
Eluate	0·17 *F*	—	0·25 *Q**	0·25 grey
	0·27 *Q*	—	0·32 *F*	—
	0·39 *F*	0·39 orange	0·40 *F*	—
	—	0·48 grey	0·49 *F*	—

TMA, TMEA and TMPA, tetramethyl-, trimethylethyl-, and trimethylpropylammonium iodide. *Q* and *F*, quenching and fluorescent reactions, respectively, in ultra-violet light. [ ] Area of eluate contracting frog rectus muscle.

* In this solvent an ultra-violet-quenching material did not clearly separate from the active substance.

pharmacological activity again appeared in both solvent systems at an R_F identical with that of TMA and reacting with iodine. It was readily distinguished chromatographically from ACh, urocanylcholine, and other quaternary ammonium compounds. The results of such an experiment are shown in Table 3.

The possibility that low concentrations of TMA exist in *M. senile*, *C. parasitica* or *Physalia* cannot be excluded, since chromatograms of their tissues show a number of iodine-reactive bands. However, since these bands when eluted do not contract the rectus muscle, and since extracts of large amounts of tissue are inactive, the concentration of active esters of choline or of tetramethylammonium must be very small.

Unidentified protein-like substance

Saline extracts of 1–10 mg freeze-dried tentacle of *A. equina* or *A. sulcata* produced a maximal, maintained contracture of the frog rectus abdominis muscle. This contracture differs from that produced by ACh or TMA in that frequently there are superimposed quick contractions (Fig. 3*b*). The contracture is also protracted, and with larger doses was apparently irreversible, the muscle failing to relax even after 90 min or longer; even with smaller doses it was usually maintained for 40 min or longer. This property made it difficult to study repeated effects on the same muscle preparation. In some instances the muscle showed repetitive quick contractions, resembling simple twitches, even after the extract was washed out. Both the contracture and quick contractions of the rectus muscle were unaffected by concentrations of tubocurarine (10^{-3} g/l.) which blocked the action of ACh, TMA, or the alcohol extract of *A. equina* (Fig. 3*a*). The effectiveness of this substance is apparent by the fact that the freeze-dried tentacle of *A. equina* contains an ACh equivalent activity of approximately 500 μg/g when tested on the frog rectus muscle. The tentacles of *A. equina* contain about twice the activity as the rest of the organism as was also found in the case of TMA. The tissues of *A. sulcata* contain approximately one third the activity of *A. equina*.

At similar concentrations to those producing contracture of the rectus abdominis muscle, this substance, like ACh, produced a quick contraction, or twitch, of the isolated frog sartorius muscle. The effect still occurred in the presence of tubocurarine (10^{-3} g/l.) when the response to ACh was completely blocked. This muscle, like the rectus, also showed repetitive, quick contractions during, or after, exposure to the extract.

The substance failed to dialyse across a cellophane membrane (24 hr, 4° C) and was insoluble in 95% ethanol, the insoluble residue being fully active when taken up in water. It was completely inactivated by heating for 5 min in a boiling water-bath in N/100-NaOH and also on incubation with crystalline chymotrypsin (500 μg/ml., 37° C, 20 min) (Fig. 3*b*). It was relatively stable, however, on heating in N/100-HCl.

Homarine (N-methyl picolinic acid betaine)

We were impressed early in our experiments by an ultra-violet-absorbing band in all our chromatograms. This band also reacted with iodine. Definite evidence that this substance was homarine was obtained by paper chromatography and by measurement of its ultra-violet absorption spectrum.

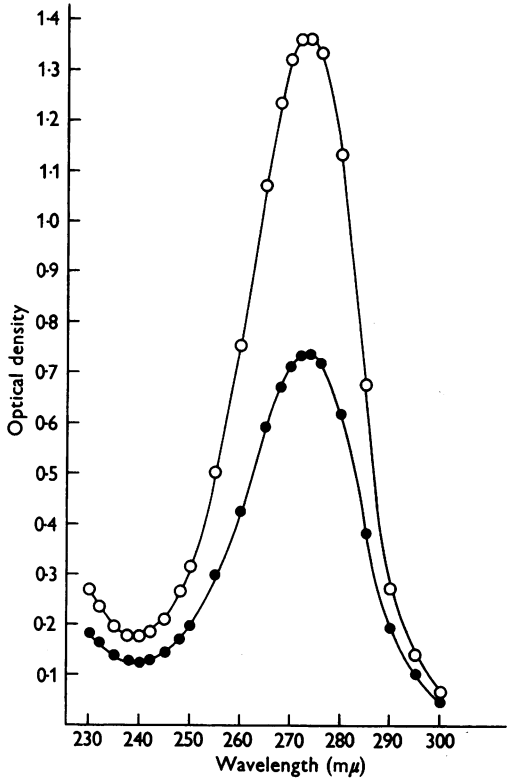


Fig. 5. Ultra-violet absorption spectra of synthetic homarine and eluate corresponding to homarine in R_F and other properties, in chromatogram of *A. equina* (body). *n*-Propanol–formic acid solvent. Eluate pH, 5.

Extracts of the anemones and of *Physalia* chromatographed in *n*-propanol–formic acid showed a strong ultra-violet-quenching, iodine-reactive area, with an R_F value identical with that of synthetic homarine. The eluate from this band on the paper in each case showed the identical ultra-violet absorption maximum and minimum values of synthetic homarine, at 273 and 240 mμ, respectively. As with homarine there was no shift in these values when the pH was adjusted with HCl or NaOH to pH 2 or 12. The concentrations of homarine were calculated by using the

molar extinction coefficient quoted by Keyl *et al.* (1957) and are shown in Table 4. They are, in general, very high, particularly in the tentacles of *A. equina* and in the coelenteric tissue of *C. parasitica*, namely 1·5 and 2·1 mg/g freeze-dried tissue, respectively.

The identification of homarine was confirmed by further procedures in the case of extracts of *A. equina*. In this instance, extracts of body (whole minus tentacle) were chromatographed on Whatman No. 4 paper in three different solvents, with synthetic homarine as a marker. R_F values of 0·36, 0·31, and 0·09 were obtained for synthetic homarine in *n*-propanol–formic acid, *n*-butanol–acetic acid, and *n*-butanol–ammonium hydroxide solvents. A band with identical R_F values, also reacting with iodine and showing strong ultra-violet-quenching properties, was obtained with the extract in all three solvents. On elution, these bands were found to have ultra-violet absorption spectra identical with that of homarine (Fig. 5); as with homarine they were unaffected by altering the pH of the solution to 2 or to 12.

Other substances

Chromatograms in propanol–formic acid of extracts of all these coelenterate tissues regularly showed a marked iodine-reacting band with a low R_F value of approximately 0·2. This area was not visible when exposed to ultra-violet light, did not react with Pauly's reagent, and the eluate was inactive on the frog rectus muscle. An apparent positive ninhydrin reaction proved to be negative when the iodine-reacting area was eluted and rechromatographed in *n*-butanol–acetic acid. In this solvent the ninhydrin-positive area separated clearly from the one reacting with iodine. Hydrolysis of the eluate (in N/10-NaOH, 98° C, 5 min) did not alter the R_F of the iodine-reacting band. This substance, therefore, is not a choline ester. It was also distinguished from betaine, which had a much higher R_F value, approximately 0·38 in the same solvent, and similarly from simple alkyl quaternary ammonium compounds which had relatively high R_F values (Table 3). It was also distinguished from γ-aminobutyric acid which had an R_F value of 0·45 in this solvent, and which, as expected, gave a strong ninhydrin reaction. This substance remains, as yet, unidentified. We have not excluded the possibility that it is γ-butyrobetaine which was identified by Ackermann (1927) in the tissues of *A. equina*.

DISCUSSION

Three of the four pharmacologically active substances found in the coelenterates studied are present in the highest concentrations in the tentacles. They are histamine, tetramethylammonium, and a toxic protein-like substance. It would seem, therefore, that they are related to

the presence of nematocysts, but their presence in relatively high concentrations in other parts of the animals indicates that they are not likely to be associated solely with these stinging cells.

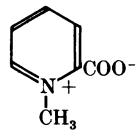
The major toxic component appears to be the non-dialysable substance, probably a protein, which produces a relatively irreversible contracture of the rectus abdominis and a twitch of the sartorius muscle of the frog. The nature of the response of the rectus muscle to this substance suggests that it activates both the slow (contracture) and quick (twitch) fibres under conditions in which cholinesters and alkyl quaternary ammonium compounds activate only the slow fibres. The fact that, like acetylcholine, it produces a twitch of the isolated sartorius muscle is consistent with this possibility, since the sartorius muscle of the frog is thought to contain fibres of the 'quick' type only (Kuffler, 1953, 1955). The remarkable activity of this substance on frog skeletal muscle suggests that an analysis of its action may be of interest. Toxic proteins with marked effects on skeletal muscle have been found recently in the salivary gland of the octopus and cuttlefish (Ghiretti, 1959) and in bee venom (Neumann & Habermann, 1954). There may be a group of such toxic proteins with an action on skeletal muscle.

There is a difference in pharmacological activity of extracts from the different anemones. Thus, whereas extracts from *A. equina* are extremely toxic, those from *M. senile* are pharmacologically inert. This difference would appear to correlate with their habits, in so far as *A. equina* is a sessile but predatory organism whereas *M. senile*, with its dense fine tentacles, is probably a passive 'filter-feeder'. Similarly, *C. parasitica*, which lives on the shell of the hermit crab, is believed to share food already captured by the crab (Orton, 1922; Stephenson, 1935).

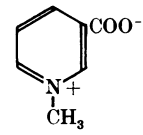
The high concentration of 5-HT in the coelenteric tissues of *C. parasitica* is in contrast with its absence in other anemones. A similar situation in regard to 5-HT exists in Octopoda, where the salivary gland of *O. vulgaris* contains approximately 500 μg/g whereas that of *O. macropus* contains none (Erspamer, 1954). Our finding that the lowest concentration of 5-HT in *C. parasitica* is present in the tentacles does not support the suggestion of Welsh (1955) that this substance is a specific nematocyst toxin in *Metridium* and in *Physalia*. Indeed, we could find no evidence for the presence of significant amounts of 5-HT in any of the tissues of these two animals. This does not support the view (Editorial, *Brit. med. J.* 1957) that the toxic reaction in man following contact with *Physalia* tentacle is in part due to the action of 5-HT.

Our results demonstrate the widespread distribution and high concentration of homarine (*N*-methyl picolinic acid betaine) (Fig. 6) in coelenterate tissues. Little has been learned about this base since it was dis-

covered by Hoppe-Seyler (1933) in the tissues of the lobster *Homarus vulgaris*, the lamellibranch *Arca noa*, and the sea urchin *Arbacia pustulosa*. Recently, however, Ackermann (1953) has identified the closely related base trigonelline (*N*-methyl nicotinic acid betaine) (Fig. 6) together with homarine in extracts of the sea anemone, *A. sulcata*, and Gasteiger (Gasteiger, Gergen & Haake, 1955) has drawn attention to the high concentrations of homarine in marine arthropods and molluscs. Homarine has no known pharmacological activity, and as yet there are no reports of its presence in non-marine or vertebrate animals. It would seem likely that a substance occurring in such high concentrations in several large invertebrate phyla is of biological significance, and it would be of interest to extend the study of its distribution in nature.



Homarine
(*N*-methyl picolinic acid betaine)



Trigonelline
(*N*-methyl nicotinic acid betaine)

Fig. 6. Structure of homarine and trigonelline.

The chemical nature of thalassine, which is the pruritogen and histamine liberator in anemone extracts, has not been solved. It has been claimed that thalassine is, in fact, tetramethylammonium (Courville, Halstead & Hessel, 1958). This cannot be so, however, since we administered varying doses of TMA to dogs, subcutaneously or intravenously, and failed to produce any sign of the syndrome described by Richet (1902). Also, TMA failed to release histamine from perfused cat skin preparations. The substance we described, with an action on skeletal muscle like Richet's congestin which produces pulmonary oedema, is probably a protein, but it remains to be determined whether they are the same or different substances.

SUMMARY

1. The identification and distribution of the pharmacologically active substances, 5-hydroxytryptamine, histamine and tetramethylammonium, were determined in four species of sea anemones and in the Portuguese man-of-war, *Physalia*, by pharmacological and chemical tests. In those species where they were identified, histamine (*Actinia equina* and *Anemonia sulcata*) and tetramethylammonium (*A. equina*) were present in the highest concentrations in the tentacles. 5-Hydroxytryptamine, however, was present in very high concentrations in the coelenteric tissues of one

species (*Calliactis parasitica*), and in much lower concentrations in the tentacles.

2. A substance, probably a protein, with an action on skeletal muscle, was present in tentacles, and to a less extent in other tissues, of *A. equina* and *A. sulcata*. It produced a maintained contracture of the frog rectus muscle with superimposed quick contractions, and a quick (twitch) contraction of the frog sartorius muscle. The high concentration of this substance and its potency suggest that it is the major toxic agent in the tentacles of these anemones.

3. Homarine (*N*-methyl picolinic acid betaine) was identified in high concentrations in the different parts of all the animals examined.

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