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# VII. A Toxic (Histamine-Releasing) Principle from Tentacles of *Cyanea capillata* (the Stinging Jelly-fish)

BÖRJE UVNÄS, *Farmakologiska Institutionen, Karolinska Institutet, Stockholm 60*

*Cyanea capillata*, otherwise known as the sea blubber, is a stinging jelly-fish which occurs in the waters of the Swedish west coast in late summer and early autumn. The jelly-fish vary considerably in size but average about 10–20 cm in diameter. The numerous tentacles may reach several metres in length and are amply provided with nematocysts.

Contact with *Cyanea* tentacles elicits an immediate cutaneous reaction. An intense burning sensation is felt at the site of contact, followed by erythema and sometimes slight oedema. Goose-flesh due to piloerection occurs within the affected skin area. The symptoms vanish within 5–10 h. Facial erythema and oedema accompanied by intense pain, cold sweats, pallor, tachycardia and weakness, and difficulty in breathing accompanied by objective signs of marked bronchoconstriction strengthen the impression that either the *Cyanea* toxin or active principles released from the affected skin can penetrate into the general circulation and cause remote tissue reactions.

Our interest in the toxicity of *Cyanea capillata* stems from our studies of the mechanism of anaphylactic (allergic) histamine release. Extracts of the tentacles were found to contain a natural principle capable of releasing histamine from skin and other histamine-containing tissues. If such principles release histamine by the same mechanism as that instrumental in anaphylactic (and allergic) reactions, they could be used as model substances for further studies of the histamine-release process.

## Preparation of Jelly-fish Material

In the collection of jelly-fish material we usually collect only the tentacles. They are cut off and allowed to drop into alcohol.

A crude but fairly active material is then prepared by repeated precipitation with ethyl alcohol. After removal of undissolved material by centrifugation, the original alcohol solution is concentrated *in vacuo* to about 1/15 of its initial volume. Alcohol is added again, this time to 67 per cent. The precipitate is discarded. On concentration *in vacuo* a large amount of salt-containing material precipitates and is removed by centrifugation. From the now rather concentrated *Cyanea* extract, further inactive material is precipitated by once more adding alcohol to 50 per cent. At 90 per cent alcohol, a precipitate forms to which the active *Cyanea* principle adheres. This precipitate is dissolved in water; inactive material is again removed by alcohol precipitation at 50 and 67 per cent, and the active material is then precipitated by alcohol in great excess. Dried *in vacuo* and stored dry at room temperature, the material remains active for years. Five litres of the original alcohol solution yields about 700 mg of dry material. Tested on mesentery mast cells, 10–20 μg/ml of this material causes disruption of 50 per cent of the cells.

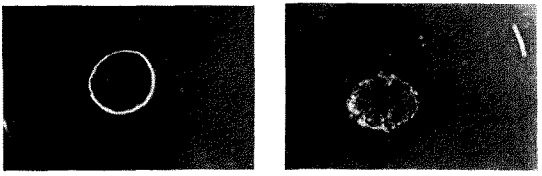
## Techniques for Mast-Cell Studies

Most tissue histamine has been found to be stored in the mast cells and this poses the question: how is the histamine released from the mast cells? This problem led us to isolate histamine-releasing principles from biologic sources, one of which was the stinging jelly-fish.

When convinced that the *Cyanea* tentacles contained a factor that released histamine, probably by attacking the mast cells, we developed a very simple technique by which mast cells can be isolated from rat peritoneal fluid,[1,2] in a modification of a previously described method.[3] Fig. 1a shows a mast cell isolated by our differential centrifugation technique. Under the water immersion lens the cell appears as a transparent, spherical body with a smooth surface. Exposure to a histamine releaser, for instance *Cyanea* extract, triggers an eruptive process in the cell. The cell first swells and intense intracellular movements occur. It then loses its smooth spherical form, taking on more the rough surface of a mulberry (Fig. 1b). The rough surface gives the impression that the intracellular granules have penetrated the

cell membrane, but due to forces of attraction they continue to adhere to the cell. Under the microscope some granules can be seen to float away from the cell surface. Sometimes the cellular process seems sufficiently violent to 'throw off' granules from the cell.

The isolated cells have a content of histamine averaging 10 μg per $10^6$ cells. In a suitable balanced salt solution, the cells retain



(a) (b)

Fig. 1. Isolated rat mast cells; (a) control, (b) exposed to histamine releaser

their histamine for hours and even days, but they release it rapidly within a few seconds on exposure to a histamine liberator.

We felt justified in concluding from our studies that the *Cyanea* principle releases histamine by activating enzymatic processes in the mast cells. The histamine-releasing action shows a narrow and distinct pH optimum. It is strongly influenced by the ionic environment. The temperature curve is rather instructive, and of especial interest is the effect of heating the mast cells. Incubation of the mast cells at 43° or above for a few minutes makes them irreversibly resistant to the influence of the *Cyanea* factor. Since the histamine-releasing principle is quite stable at this temperature, the observation agrees with the supposition that the acquired resistance is due to inactivation of a cellular enzyme system. It was further observed that enzyme inhibitors, believed to act by attacking $NH_2$ and SH groups in 'specific' concentrations, block the effect of *Cyanea* extracts. The mast-cell degranulation and the histamine release were completely blocked by metabolic inhibitors such as dinitrophenol, thyroxine, sodium azide, cyanide, etc.

The data presented may suffice to illustrate our findings, which suggest that the *Cyanea* principle initiates a rather complicated energy-requiring process in the mast cells, possibly involving the active transport of granules (and histamine) to the cell surface. *If so*, the nearest equivalent to such a process seems to be secretion. Anyhow, our studies of the *Cyanea* principle have led us to fundamental problems in cellular physiology and intracellular transport.

We have not yet had the opportunity of studying directly the action of the *Cyanea* principle on human mast cells. Human skin is rich in such cells, especially around the ducts of the sweat glands and the hair roots. The active principle seems to penetrate the skin rather easily, as shown by the fact that a drop of active *Cyanea* material produced an almost instantaneous burning sensation with local erythema and weal formation. It seems reasonable to assume, therefore, that the symptoms produced on contact with *Cyanea* tentacles are due to the action of a histamine liberator introduced into the skin.

Not only histamine but other substances stored in the mast cells will be released on degranulation of mast cells. Mast cells from some animal species, e.g. the rat, contain serotonin.

The nematocysts of the *Cyanea* tentacles are believed to deliver the poison by which the animal kills its prey. The questions of whether the jelly-fish sting is due to the same poison and whether the histamine-releasing agent comes from the nematocysts at all cannot be answered as yet. There are observations suggesting that the histamine-releasing agents may be localized outside the nematocysts.

Histamine-releasing extracts can also be obtained from various lower organisms. Richet[4] extracted from nematocyst-containing animals, such as the sea anemone, and from the lobster, oyster and other shellfish, fractions that, when injected intravenously in dogs, produced urticaria and itching, and in high doses resulted in shock-like conditions and death. Our initial studies on *Cyanea* more or less duplicated the extraction technique recommended by Richet for obtaining thalassin, the name he gave the active principle in the sea anemone tentacles. The extracts which we made according to Richet's description were highly active.

Our most active histamine-liberating agent, however, was

obtained not from a marine organism but from hog and horse *Ascaris*.[5] Extracts from these round worms yielded products that showed a considerable histamine-releasing activity in concentrations below 1 μg/ml.

Chemically, the principles from *Cyanea* and *Ascaris* were both fairly resistant to heat. They were soluble in hot absolute alcohol and withstood boiling in 0·5N hydrochloric acid for at least 30 min. After adsorption on charcoal or Amberlite IRC 50 (XE 64), a material was obtained that was active in microgram quantities. Careful hydrolysis of this material in hydrochloric acid removed much ninhydrin-positive material, but even when hydrolysis was continued to the point of incipient inactivation it still gave strong ninhydrin and sugar reactions. Paper chromatography indicated the presence of numerous amino acids (as yet undefined).

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