Isolation of Active Nematocysts of *Metridium senile* and their Chemical Composition

During the past half-century, there has been recurrent interest in the chemical nature of the stinging capsules of cœlenterates. Mainly two lines of investigation have been pursued. Toxic extracts of the tentacles or whole animal have been examined[1], and histochemical studies have been made. Both these approaches have yielded interesting information for speculation, but as Hyman[2] has pointed out, "On the whole it must be concluded that the nature of the nematocyst toxin is unknown, since the extracts mentioned above include tissues as well as nematocysts . . .", and one cannot expect the histochemical approach to yield the definitive answers.

Since the toxins of nematocysts would be useful substances for testing the immune antitoxic responses of a variety of marine invertebrates, studies were undertaken to find a way of isolating active nematocysts in a purified form and in sufficient quantity to allow chemical analyses and the isolation of the toxin. A method has been found of doing this, and it has afforded some information about the chemical nature of the wall and internal contents of the nematocysts of *Metridium senile*.

Briefly, the method involves maceration of the whole anemones in a Waring blendor with an equal volume of 1 *M* sucrose in sea water (maceration in sucrose solution was originally suggested by Miss Eleanor Dodge, Zoology Department, University of Washington). The creamy suspension is passed through graded screens with openings of 1·168, 0·589, 0·295, and 0·147 mm. with the aid of suction. Tyler standard screens customarily used for geological investigations were fitted on to a Buchner funnel with the aid of masking tape. The suspension after this filtration consists of nematocysts, fine tissue debris, dissolved tissue constituents and very fine sand. The amount of sand can be greatly diminished by keeping the anemones on a wire screen for several days before macerating.

Centrifugation of the suspension at approximately 1,000 rev./min. for fifteen minutes collects the nematocysts, and they are washed with additional sucrose solution to free them of soluble materials. Differential centrifugation in a small International clinical centrifuge finishes the purification. 15-min. centrifugations are used to collect the nematocysts and free them from fine tissue fragments. 15-sec. centrifugations are used to remove the sand. After five or six such centrifugations, the material appears to be all but completely free of both the sand and tissue fragments when examined microscopically, and is chemically consistent from batch to batch with respect to total nitrogen and hexosamine content. The nematocysts may be discharged with distilled water, dilute acid or base, sodium thioglycollate, or methylene blue, and the material released is toxic to those animals tested: the barnacle, *Balanus glandula*, and the snail, *Littorina planaxis*.

Chromatographic analyses of hydrolysates of the walls and tubes after discharge have revealed the presence of eighteen amino-acids: cysteine, aspartic acid, glutamic acid, serine, glycine, histidine, lysine, arginine, threonine, alanine, tyrosine, proline, valine, methionine, phenylalanine, leucine, isoleucine, and tryptophan. Two-dimensional chromatograms were developed with phenol/water (80 : 20), followed by butanol/acetic acid/water (4 : 1 : 5). Isatin and ninhydrin were used for the detection of spots. In addition, a hexosamine resembling galactosamine and a uronic acid resembling glucuronic acid were detected in acid hydrolysates through the use of one-dimensional chromatograms with ethyl acetate/pyridine/water (2 : 1 : 2) and *p*-anisidine hydrochloride or ninhydrin for the detection of spots. Quantitative determinations[3] of these carbohydrates has revealed that they are present in an equimolar ratio which may indicate the presence of chondroitin sulphuric acid; a polysaccharide similar in solubility characteristics has been isolated. These findings suggest that the walls may be formed of a cartilagenous material.

The material released on discharge of the nematocysts in distilled water shows on hydrolysis the same amino-acids, with the exception that tyrosine and tryptophan appear to be lacking. The same hexosamine and uronic acid are present, but in this material they are in a molar ratio of four hexosamines to five uronic acids. It is still uncertain whether the internal mucoproteins are of more than one species. In addition to the mucoprotein material, two hydroxyindoles have been detected. One of these, which is extractable with ether from acidified material, resembles 5-hydroxytryptophan with regard to its colour reactions and fluorescence. The other, which is extractable with ether from basic solutions, resembles bufotenine (N dimethyl 5-hydroxytryptamine) with regard to chromatographic development in basic, neutral and acid solvents. In addition to giving the bluish Ehrlich reaction, it fails to give the greenish-yellow colour of 5-hydroxytryptamine with ninhydrin in acetone. This latter substance has recently been found in tentacle extracts of *Metridium*[1]. Further work is under way on the identification of these substances.

The analyses, which have been carried out to this point, indicate that the material injected into the prey of these anemones is a mucoprotein with either adsorbed or free hydroxyindoles. It is hoped that through the investigation of purified suspensions of nematocysts their nature will be revealed.

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Larvæ of the Polypterid Fish *Erpetoichthys* Smith

When J. S. Budgett studied the *Polypterus* larva in the Niger Delta in 1903, he also searched for larvæ of the closely related *Erpetoichthys*, but did not find any. Apparently later workers have been equally unsuccessful, since there seem to be no records of juvenile *Erpetoichthys*. It was assumed that *Erpetoichthys* would possess a larval stage similar to that of *Polypterus*, with pinnate external gills; but this had not so far been confirmed.

On March 17, 1956, towards the end of the dry season, I caught two young specimens of *Erpetoichthys* at Aiyetoro about 40 km. west-north-west of Abeokuta in south-west Nigeria, both still in the larval condition. The find was made at the point where a small bridge on the Iboro Road crosses a

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