AD-A054 925 PROFESSIONAL STAFF ASSOCIATION OF LAC/USC MEDICAL CEN--ETC F/G 6/20
BEHAVIOR, ECOLOGY AND TOXICITY OF VENOMOUS MARINE FISHES.(U)
DEC 77 F E RUSSELL N00014-76-C-0254

UNCLASSIFIED

1 OF 1
AD
A054 925

END
DATE FILMED
7-78
DDC

FOR FURTHER TRAN

AD 770 980

PROFESSIONAL STAFF ASSOCIATION Los Angeles County/ University of Southern California Medical Center

(12)

(11) 31 Dec 77 (12) 19p.

AD A054925

TITLE: (6) Behavior, Ecology and Toxicity of Venomous Marine Fishes.

NAME OF AUTHOR: (10) Findlay E. Russell

REPORT: (9) Final Technical Report.

PROJECT PERIOD: 1 Oct 1975 - Dec 31 1977

CONTRACTOR: Professional Staff Association of
The Los Angeles County/University of
Southern California Medical Center
Los Angeles, California 90031

ONR CONTRACT: (15) N00014-76-C-0254

TASK NUMBER: NR 104-793

PSA PROJECT: 9-169-0-0

AD No. DDC FILE COPY

Reproduction in whole or in part is permitted for any purpose of the United States Government.

Distribution of this document is unlimited.

DDC RECEIVED MAY 24 1978 E

406 790

1739 GRIFFIN AVENUE • LOS ANGELES, CALIFORNIA 90031 • TELEPHONE (213) 223-4091

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|---|
| 1. REPORT NUMBER<br>#9 & #10 | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE (and Subtitle)<br>Behavior, Ecology and Toxicity of Venomous Marine Fishes | | 5. TYPE OF REPORT & PERIOD COVERED<br>Technical Report<br>10/1/77 to 12/31/77 FINAL |
| | | 6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s)<br>Findlay E. Russell, M.D. | | 8. CONTRACT OR GRANT NUMBER(s)<br>NR 104-793<br>N00014-C-0254 P000 2 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>Professional Staff Association' of the LAC/USC Medical Center<br>1739 Griffin Ave., Los Angeles, CA. 90031 | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS<br>NR 104-793<br>N00014-C-0254 P000 2 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS<br>Ocean Science and Technology Division<br>Office of Naval Research<br>Department of the Navy-Arlington, VA. 22217 | | 12. REPORT DATE<br>12/31/77 |
| | | 13. NUMBER OF PAGES |
| 14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) | | 15. SECURITY CLASS. (of this report)<br>None |
| | | 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE |

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release - Distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Same

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

When the crude venom is fractionated by gel filtration chromatography, the lethal activity is associated with proteins having a molecular weight of more than 40,000. From studies with Sephadex G-200 and Bio-Rad P-300 the lethal activity would seem to have a molecular weight of less than 2,000,000. Following separation on Sephadex G-200, the semi-purified lethal fraction has an IV LD 50 in mice of 0.9 mg/protein;kg body weight, while the LD 50 of the crude venom is 2.6 mg/protein/kg. The venom can also be separated by ion exchange chromatography but no lethal material is detected on DEAE-C.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE
S/N 0102-014-6601

None
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

## INTRODUCTION

Our Office of Naval Research program (Contract No. N00014-76-C-0254) at the University of Southern California, Los Angeles County/University of Southern California Medical Center has been a highly successful and productive research endeavor. Through the course of the contract periods a considerable amount of basic knowledge has been obtained. This knowledge has been of importance to the biomedical community, as well as to the Navy. In addition, an encouraging amount of applied data have been obtained, much of which is being utilized by the Navy or by other governmental and private agencies.

The success of the program, as reflected by the more than 60 papers and 200 presentations by members of the staff over the past decade, can be attributed to the continuous ONR interest in a discipline in which long-term support is essential. The importance of these research programs to the Navy is reflected by the numerous inquiries the applicant receives from senior Navy officers throughout the world.

## PROGRESS TO DATE

During the past 27 months, we have concerned ourselves with four general areas: 1) the general biology of certain venomous marine fishes of nuisance importance to Navy personnel; 2) the chemistry and electron microscope appearance of the skin of certain fishes which have been found to be toxic; 3) the ultrastructure of the venom apparatus of the stingrays and scorpion fishes; and 4) the chemistry and pharmacology of stingray and scorpionfish venom.

(cont on p. B)

White Section
Buff Section
JUSTIFICATION
BY
DISTRIBUTION/AVAILABILITY CODES
DIST. AVAIL. and/or SPECIAL
A

We have also continued to evaluate the modes of treatment for ciguatera poisoning. There is evidence to indicate that during recent French Navy manuevers in the Pacific, some navy personnel were stricken with ciguatera poisoning. This entity is becoming of considerable importance to navy personnel, as well as to food experts, commercial fishermen, physicians and public health workers in the Pacific.

Further work on the chemistry of the venom of the California scorpionfish indicate that sulfhydryl groups are required for the lethal activity of the venom. Cystine and reduced glutathione appear to have no significant effect on this activity until several days have passed. Cleland's reagent, an effective sulfhydryl reagent, stabilizes the lethal activity. The lethal activity is sensitive to heat, oxidizing agents and extremes of concentration and pH. The venom contains nonsignificant amounts of proteolytic and phosphodiesterase activity.

When the crude venom is fractionated by gel filtration chromatography, the lethal activity is associated with proteins having a molecular weight of more than 40,000. From studies with Sephadex G-200 and Bio-Rad P-300, the lethal activity would seem to have a molecular weight of less than 2,000,000. Following separation on Sephadex G-200, the semi-purified lethal fraction has an IV $LD_{50}$ in mice of 0.9 mg/protein/kg body weight, while the $LD_{50}$ of the crude venom is 2.6 mg/protein/kg. The venom can also be separated by ion exchange chromatography but no lethal material is detected on DEAE-C.

Cellulose acetate strip electrophoresis studies indicate that the semi-purified lethal fraction obtained from Sephadex G-200 consists of

more than one fraction. The lethal fraction is associated with moderately negatively-charged components of large molecular weight. Studies employing immunoelectrophoresis failed to show any significant protection against S. guttata venom by the commercially prepared antisera prepared against stonefish venom.

The venom of the scorpionfish Scorpaena guttata has a primary muscarinic action on the rat atria and causes a secondary beta adrenergic stimulation. The primary effect is probably due to release of endogenous acetylcholine. The venom does not appear to contain acetylcholine-like components but causes the release of endogenous acetylcholine. The secondary positive venom response can be inhibited by either a beta adrenergic blocking agent or by depleting endogenous norepinephrine with reserpine. The venom appears to release both endogenous acetylcholine and catecholamines, although the mechanisms appear to be separate. The venom produces few changes in the guinea pig phrenic nerve-diaphragm preparation. It has a mild, direct hemolytic effect, in vitro, but does not affect the blood clotting systems, nor does it appear to produce significant hemolysis in the dog.

Scorpionfish venom produces an immediate hypotensive crisis, when injected intravenously, although pressure returns to control values within 60 seconds of the injection. During this period of arterial hypotension the heart rate remains stable but bradycardia develops about 30 seconds after the lowest peak of the crisis is reached. The decline in arterial pressure may result from action on both the myocardium and peripheral vasculature, both of which were demonstrated. Changes in the pulmonary

arterioles were also observed and their mechanisms studied.

Studies on the venom secreting tissues of S. guttata indicate that the principal secretory cells correspond in detail to the eosinophilic cells described by Russell and Lewis (1956). The nuclear profiles are slender and contorted, strikingly different from the deeply-stained ovoid nuclei of the interposed connective tissue cells, which extend in strands between the basal surface of the secretory epithelium and the superficial epidermal cell layer. The cytoplasm of the large secretory cells is optically homogeneous, except for two conspicuous types of inclusion. The first consists of small unstained vesicles, generally arranged in groups, and always much smaller than the nucleus, ranging in size down to the limits of resolution of the light microscope. The second type of inclusion is circular or ovoid in profile, often larger than the nucleus and occurring either singly or in small numbers within each cell.

Electron micrographs reveal that the elongated nucleus is set in a finely grandular cytoplasm. The fine cytoplasmic vacuoles are membrane-limited, containing a variety of cellular debris and are evidently "isolation bodies" or cytolysomes. The larger inclusions, seen as lightly stained bodies in the light microscope, are conspicuous in electron micrographs of these cells; they are revealed as membrane-limited vacuoles, several micra in diameter, filled with microtubular profiles. Morphologically similar components are often seen in animal and plant cells, and hitherto been described as either intranuclear (associated with the division spindle) or free within the cytoplasm, but never sequestered

within a membrane-limited cytoplasmic compartment. Within the vacuole, the microtubules are curved, and present a swirling pattern, conforming to the spheroidal limits of the enclosing vacuole. Within each vacuole, the concentration of microtubular profiles is greater than in any other source, and a vacuole of average diameter (7 μm) was estimated to contain 50,000 profiles.

In the skin of the soapfish Grammistes sexlineatus, three secretory cell types were found to be present with distinctly different cell contents. The first cell type (SC1) is elongated and contains abundant homogeneous, "glassy" eosinophilic material. This material is PAS and Sudan Black negative and the cell does not appear to correspond to the mc 1 or mc 2 cells of Randall et al. The second cell type (SC2) is smaller, concentrated at the surface of the epidermis and also contains eosinophilic homogeneous material, but this is PAS positive and SB negative. This cell is the mc 1 cell of Randall et al. The third cell type (SC3) is similar to the coarsely granular contents which are PAS negative and SB positive. It appears to be the mc 2 of Randall et al.

The multicellular dermal glands empty to the surface via a duct. The lining cells of the duct are similar to the elongated epithelial cells of the epidermis and are best described as squamous. In gland and duct are also vacuolated cells which contain PAS negative, SB positive material. The glands seem to be limited to the upper dermis, above the scale insertions. We have designated these glands as secretory glands, type 1 (SG1).

The multicellular glands, contain eosinophilic material in the deep dermis between scale insertions. This second type of gland (SG2) also

contains PAS negative, SB positive material. Although it is possible that SG1 is a duct leading from the deeper multicellular glands (SG2), we have found no connections between the two. However, we have observed thin-walled ductile structures arising from the deeper dermis.

A low-power electron micrograph of the epidermis shows three types of secretory cells. It is obvious that the three epidermal secretory cells have distinctly different contents. In addition, SC3 cells contain a range of different sized granules, suggesting a sequential development from fine granular material into larger aggregates. Based on the granule size, we have labeled these cells SC3a, b and c. SC1 cells also show different types of granules. SC1a cells contain smooth finely granular contents, while SC1b are tightly packed with minute spheres with electron-lucent centers. The contents of SC2 are quite different. The material is quite electron dense and appears to be contained in spherical packets which are compressed and flattened by crowding.

PUBLICATIONS


1) Russell, F.E. Pharmacology of toxins of marine origin. In, International Encyclopedia of Pharmacology and Therapeutics. Section 71, Vol. 2, (H. Raskova, ed.) p. 3-114, Pergamon Press: Oxford.

2) Russell, F.E. Venom Poisoning. Bulletin of Supplementary Material in Clinical Toxicology. Univ. of Rochester, 15, 14-23.

3) Russell, F.E. and Carlson, R.W. Marine organisms. In, Biology Data Book. Altman, P.L. and Dittmer, D.S. (eds.), p. 726-735, F.A.S.E.B.: Washington, D.C.

4) Russell, F.E. Prevention and treatment of venomous animal injuries. Experientia 30, 8-12.

5) Smith, D.S., Cayer, M.L. and Russell, F.E. Membrane-limited microtubular aggregates in the venom secreting cells of a stingray. Toxicon 12, 331-335.

6) Russell, F.E. and Carlson, R.W. Jellyfish stings. In, Current Therapy. Conn, H.F. (ed.), p. 835-836, W.B. Saunders Co.: Philadelphia.

7) Russell, F.E. Poisonous and venomous marine animals and their toxins. Ann. N.Y. Acad. Sci. 245, 57-64.

8) Russell, F.E. Ciguatera poisoning: a report of 35 cases. Toxicon 13, 383-385.

9) Russell, F.E. Venomous bites and stings. In, The Merck Manual. 13th Edition. Berkow, R. (ed.), Chapter II, p. 1982-1993, Merck, Sharp and Dohme Research Laboratories: Rahway, New Jersey.

10) Chao, R.L.C., Parker, J.W., Russell, F.E. and Hashimoto, Y. Ultrastructure of the skin of the soapfish Grammistes sexlineatus. In, Toxins: Animal, Plant and Microbial. Rosenberg, P. (ed.), p. 499-508, Pergamon Press: Oxford.

11) Smith, D.S., Cayer, M.L., Russell, F.E. and Rubin, R.W. Fine structure of stingray spine epidermis with special reference to a unique microtubular component of venom secreting cells. In, Toxins: Animal Plant and Microbial. Rosenberg, P. (ed.), p. 565-582, Pergamon Press: Oxford.

12) Russell, F.E. Animal venoms. In, Practice of Medicine. Wolber, P.G.H. (ed.), Vol. IX, Chapter 30, p. 1-23, Harper and Row: Hagerstown, Maryland.

13) Russell, F.E. Terrestrial and marine venomous snakes. In, Forensic Medicine. Tedeschi, C.G., Eckert, W.G. and Tedeschi, L.G. (eds.), Vol. III, Chapter 69, p. 1437-1444, W.B. Saunders Co.: Philadelphia.