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Marine Pharmaceuticals

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The ocean has often been considered as the "mother" of all organisms providing an environment within which an almost infinite number of genetic permutations has occurred ultimately leading to the existing array of evolved forms now found in it. At one time, abiogenesis held sway since a multitude of complex random reactions could have occurred in the proposed prelife "primordial soup." But today, the results of these reactions, the organisms in the sea themselves, have taken on the major task of biogenesis in their diverse biochemical habits, assimilating simple organic molecules and building them into systems of enormous complexity (1). A consideration of the biomedical and pharmaceutical aspects of some of these interesting compounds as potential drugs and pharmaceutical adjuncts is the subject of this review.

One of the major purposes of this paper is to make the pharmaceutical community aware of the enormous potential which the sea holds as a source of new and different pharmaceuticals of all types. It will become evident that a major difficulty exists in promulgating new discoveries in this field. At least a dozen different disciplines have contributed to unraveling some of the biomedical mysteries of the deep and the results have been published in hundreds of various journals. Due to the publications explosion few people in any one discipline can cope with and keep up with new facts as they become available. Further, bits of information here and there permit only a sparse awareness of overall developments. With the recent publication of reviews of this type, aimed at different scientific groups, some of these difficulties are being overcome (2, 3).

By far the most ambitious undertaking is the definitive monograph now in its final stages of completion by Halstead (4). It is entitled, "Poisonous and Venomous Marine Animals of the World," and is a three-volume

series covering a 22-year survey of world literature from 3000 B.C. to the present. The material is covered in a taxonomic fashion and covers the history, biology, morphology, toxicology, pharmacology, and chemistry of all known poisonous and venomous animal marine organisms. This tome will go a long way in aiding and promoting investigations in all the medical and paramedical disciplines.

There also exists a number of basic reviews and popular articles which have helped set the stage for this review. Many of the basic ideas, principles, and background material are condensed from these widely scattered publications and references (5–35). Finally, it should be noted that this review originated through interest generated at a recent conference on the subject of "Drugs from the Sea," sponsored by the Marine Biology Committee of the Marine Technology Society together with the College of Pharmacy at the University of Rhode Island and the Bio-Instrumentation Advisory Council of the American Institute of Biological Sciences.[1] This meeting was held at the University of Rhode Island, August 27–29, 1967, and this review is based on an expansion and revision of a paper entitled "Current Status of Drug Compounds from Marine Sources," presented there by the author.

In order to discuss the topic under consideration, it will be necessary to define a number of important terms. First, the term poisonous is used here in the generic sense, and pertains to both oral and parenteral poisons, the former connotation being more common. However, the term, biotoxicity, is more commonly used in reference to poisonous terrestrial and marine organisms. Two major subdivisions are the plant poisons or phytotoxins and animal poisons or zootoxins. The route of administration of these gives us the further classifications of oral poisons for those which are toxic on ingestion and parenteral poisons for those administered *via* some venom apparatus. Another class, peculiar to marine organisms are those endogenous poisons found in certain glands without any structures to deliver them. These may be released *via* pores or injury to the animal. The oral poisons are generally small molecules, while the venoms are complex mixtures of enzymes which facilitate penetration, local histamine-releasing agents, pain-producing materials, and large molecular weight peptides or proteins which are the true toxic principles.

Location of poisonous materials in the organism itself gives us the terms: ichthyotoxism (fish poisoning), ichthyosarcotoxic (toxin in flesh), ichthyohemotoxic (toxin in blood), ichthyootoxic (toxin in gonads), and ichthyocrinotoxic (toxin in glands of skin). Certain terms are used to describe the syndrome or characteristic pharmacological effects of undefined poisons. Hence, the terms ciguatera poisoning caused by ingestion of a large variety of tropical marine reef or shore fishes, characterized by nervous disorders and gastrointestinal disturbances and capable of causing death; tetraodon poisoning following ingestion of certain puffers and ocean sunfish characterized by deleterious neuromuscular, respiratory, and central nervous system effects and causing about 60% mortality in victims ingesting fish in this group (here tetrodotoxin has been identified as the active toxic principle); scombroid poisoning following ingestion of the mackerel-like fish and characterized by central nervous system effects, burning of the throat, numbness, thirst, and generalized urticaria; clupeoid poisoning following ingestion of certain herring-like fish of the tropical Pacific and causing symptoms slightly different from those of ciguatera poisoning; cyclostome poisoning following ingestion of the slime and flesh of certain lampreys and hagfish and characterized by gastrointestinal distress; elasmobranch poisoning due to ingestion of shark musculature or livers and characterized by nausea, vomiting, abdominal pain, headache, diarrhea, oral parasthesia, muscle cramps, and respiratory distress; paralytic shellfish poisoning caused by ingestion of the flesh of molluscs, *e.g.*, mussels and clams, which have become toxic as a result of their ingestion of microscopic dinoflagellates and characterized by muscular paralysis; hallucinatory fish poisoning produced on ingestion of certain mullets and goatfish and characterized by nightmares and hallucinations.

Figure 1 portrays a simplified overall taxonomic diagram with one example of a biomedically interesting organism in each phylum.

HISTORY

With this brief introduction in mind, it will be instructive to review briefly the history of marine biotoxicology in order to gain some insight into how information developed in this field and why the time is long overdue for major discoveries even though the capabilities of solving some of the problems have existed for some years now. The use of natural products in one way or another is as old as mankind itself and early fact and fancy are thoroughly mixed. However, the recorded history of Babylonian, Assyrian (several thousand years B.C.), and Egyptian times (1900–1200 B.C.), particularly in the papyrus records of the latter, gives us notes on animal poisons and bites, including reference to the poisonous puffer. The Bible and Talmudic records document the toxic effects of dinoflagellates (Exodus 7:19–21; 1491 B.C.) and also the avoidance of scaleless fish (Deuteronomy 14:9–10; *ca.* 1451 B.C.) in the diet. The latter still holds true as a good rule of thumb.

The Persian and Indian writings indicate some indirect connections with the Babylonian lore and the records of Súsruta and his Súsruta-sámhita lists some 760 medicinal plants, and mentions venomous animals and antidotes for bites and stings.

In China, the recorded observations of the emperor Shen Nung (*ca.* 2700 B.C.) list 365 drugs in the Pen Tsao herbal. In "Chinese Materia Medica of Fish Drugs," Read (36) lists at least 10 toxic fish.

It was left to Grecian times (4000 B.C.) to see the beginnings of the dissociation of medicine from magic and religion. Hippocrates (460–361 B.C.) was one of the first to establish some scientific basis for medicine. Aristotle described in his writings at least 500 kinds of animals and was familiar with jellyfish and scorpion fish toxicities. That deliberate poisoning was much in vogue

[1] "Drugs from the Sea," H. D. Freudenthal, Ed., *J. Ocean Technol. Marine Technol. Soc.*, **1968**, 1.

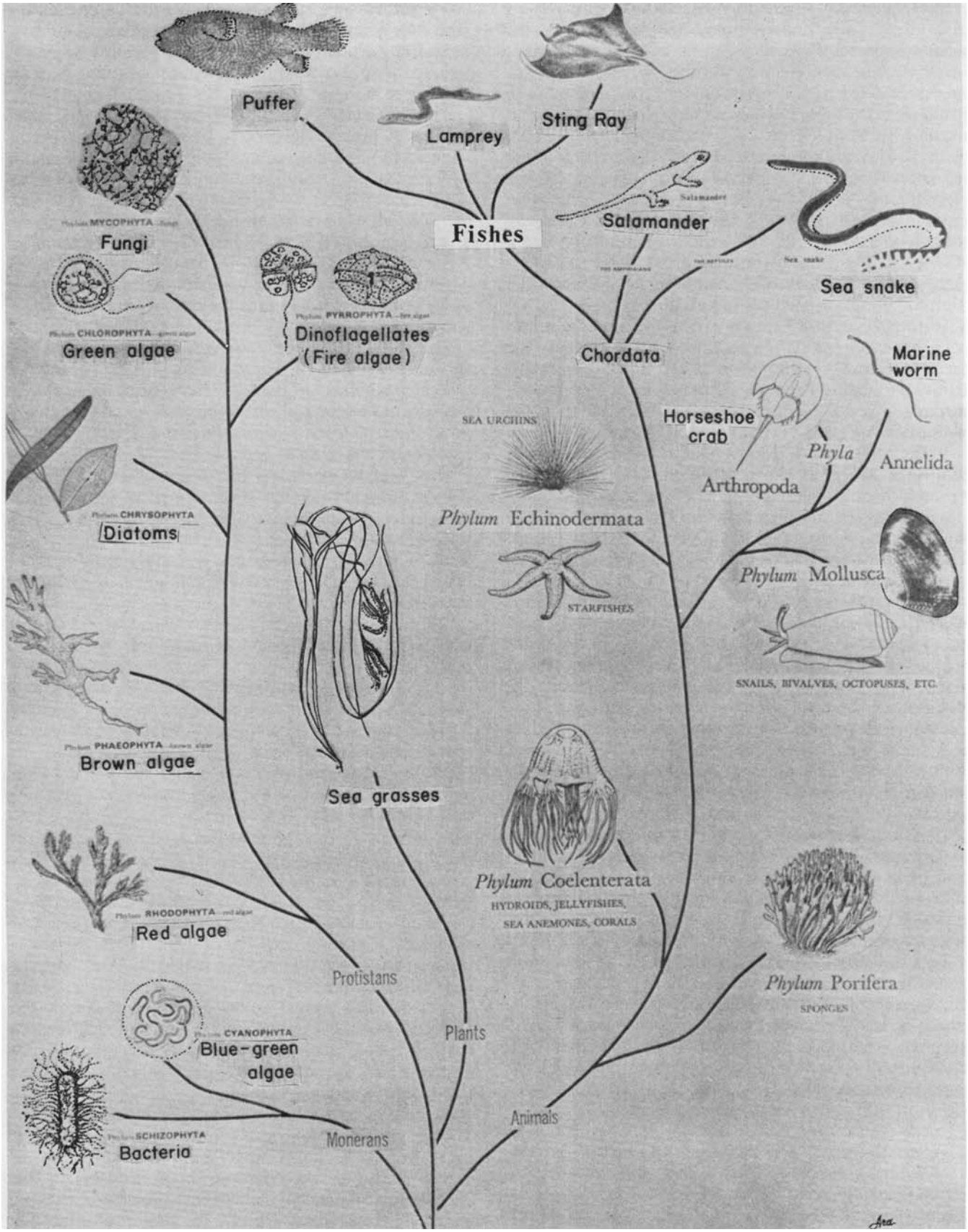


Figure 1—*Taxonomic diagram of biomedically interesting organisms.*

during these times is evidenced in the writings of Mithridates VI, king of Pontus, who was truly a royal toxicologist. He experimented with various poisons on himself and on human prisoners.

Subsequent development of the Roman empire saw early dominance in mythology, and the birth of the scientific approach to medicine is considered to have taken place around 293 B.C. when the Greek, Asclepius, was summoned to help overcome a severe epidemic in Rome. Pedianos Dioscorides, a Greek army surgeon who served with Nero (54–68 A.D.), wrote on many terrestrial plants used as drugs but also recorded the toxic nature of sea hares, polychaete bristle worms, stingrays, and sea vipers. Later, Pliny the Elder (29–79 A.D.), the great Latin naturalist, wrote on the use of ground stingray stinger as a toothache and obstetrical remedy. Galen (131–201 A.D.), who is considered the father of experimental physiology, wrote voluminously and authored over 400 books on medical subjects.

During the Byzantine empire (476–732 A.D.) the center of medical culture shifted to Constantinople where the early literature of Rome and Greece was preserved. Paulus of Aegina (625–690 A.D.) wrote an Epitome on medical subjects, the fifth book of which was concerned with toxicology and venomous stings and bites.

In the early years of the formation of the Islamic empire after Mohammed's death (632 A.D.), the Arabs, through their conquests, became acquainted with many classical writers and thereby assimilated and developed much of the earlier Greek and Roman philosophies. They wrote extensively on poisons, the effects of venoms, and antidotal remedies.

In the medieval period after the downfall of Rome, Western Europe entered into a period of intellectual decadence known as the Dark Ages. From the ninth through the fifteenth centuries the chroniclers record many dismal accounts of this era of ecclesiasticism, feudalism, pedantry, bigotry, and cruelty. Although biotoxicological knowledge stagnated through the middle ages, the medical texts of Avicenna (some 200 books and treatises) were quite popular and enjoyed wide use. In this period, Peter of Abanos wrote, "De Venenis," which treated on the nature of poisons.

The modern period is considered to have begun with the invention of printing and the subsequent rapid diffusion of knowledge. In the period from 1500–1699, the philosophical and scientific thought of such intellectual giants as Bacon, Bruno, Copernicus, Da Vinci, and others, become widely known and disseminated. With respect to biotoxicological knowledge, the toxic marine organisms in the Mediterranean region became well-known and documented. This included the weeverfish, stingrays, sea hares, jellyfish, scorpion fish, moray eels, fresh water barbels, and others. In addition, paralytic shellfish poisoning was recognized, a few venomous sea snakes were recorded, and an account of polar bear liver ingestion with toxic results was documented.

In the 18th century which followed (1700–1799), the environs beyond the Mediterranean Sea became known largely through great voyages on which physicians, naturalists, explorers, missionaries, and historians recorded their observations. The science of taxonomy came into its own largely through the systematics developed by Linné. For the first time, names of organisms were stabilized and errors of duplication in naming diminished. This enabled reliable scientific communication to take place. Through several classic papers, fish poisoning became recognized as a valid clinical entity and the symptoms and specific causative organisms definitively established. It was in this period that ciguatera, tetraodon, and paralytic shellfish poisoning became clearly recognized.

The 19th century (1800–1899) saw the clear shift from the era of natural philosophy to modern experimental science. In addition, because political freedom became widespread, a natural rebellion against metaphysical dogma occurred. The time was right and the stage set for the windfall of ideas generated by such scientific giants as Lamarck, Goethe, Schleiden, Schwann, Helmholtz, Pasteur, Mayer, Liebig, and Darwin. In this climate, experimental morphological venomology, and the beginnings of chemical isolations and pharmacological studies, thrived.

The early part of the 20th century brought the widespread publishing of experimental research based on critical analytical procedures. Here, also the budding field of immunological research led to the discovery of the hypersensitivity phenomenon and a better understanding of anaphylaxis and allergies.

Necessity, being the mother of invention and discovery, found in World War II an immediate and pressing need to further knowledge of biotoxicology. The worldwide distribution of the military into all quarters of the earth under all conceivable difficult environmental situations, brought the need for immediate first-hand knowledge to the forefront. A further demand and need for nutrients and new therapeutic agents fostered the accumulation of much useful information. However, some of the crash programs to provide fast biotoxicological information yielded spurious information indicative and characteristic of short-term sporadic research. Because the Japanese had a closer acquaintance with marine biotoxicological organisms, their war-time manuals on these subjects were far superior and still stand as remarkably good examples of proper scientific documentation (4).

Even with all the apparently continuous research on marine toxins through the various periods of history given here, most of the studies have been and continue to be regarded by the scientific community as exercises in scientific curiosity rather than anything of practical significance. Certainly, experience with terrestrial organisms, particularly plants, has taught us that many of our existing synthetics are based on molecules originally obtained from nature. A few examples, including the synthetic vasoconstricting drugs based on ephedrine, the synthetic local anesthetic based on cocaine, and the synthetic analgesics based on opium derivatives, stress this point.

According to a recent survey by Gosselin (37) over 47% of all new prescriptions filled contained a drug of natural origin as the sole ingredient, or as one of two or more ingredients. A recent review by Farnsworth (38) on the "Biological and Phytochemical Screening of Plants" has 854 references and attests to the continued search for useful drugs from terrestrial plants. Such should be the condition of research on marine organisms. With all the knowledge and experience gained in studies on terrestrial organisms, it should be quite obvious that continued research in this field of biomedical oceanology

will yield much useful information in nutrition, medicine, chemistry, military science, and drug development (18).

CHARACTERISTICS OF MARINE ENVIRONMENT

Physical Facts of Biomedical Interest

It is well known that the oceans cover nearly three-fourths or about 71% of the earth's surface. The volume of the oceans is about 325 million cubic miles with a mass of 1,560 million billion tons. They cover a surface area of 362 million square kilometers (141 million square miles) and have an average depth of water mass about 2.35 miles. Further estimates are given that 85% of the food produced by *all* plants is produced by marine plants. This is surprising when one considers that the vegetable matter grows only in the upper 150 or so feet at an estimated 4,000 tons per square mile (39).

The biomedical implications of these facts are obvious when one considers the number of organisms living in the ocean and their means of obtaining useful nutrients from it. According to Fox (1), some 1.5×10^{12} tons of suspended organic matter serve directly or potentially, *via* bacteria, as a raw food source in the perpetual metabolic cycles of the marine world. Some organisms work hard to obtain these nutrients, *e.g.*, a small colony of 10^6 mussels can filter in a year the leptopel from 22×10^6 tons of water (*ca.* 1 mile square by 25 ft. deep). Other marine organisms ingest the mussels, and in turn, are ingested themselves.

Biological Facts of Biomedical Interest

It has been estimated that about four-fifths of the earth's animal life (over 500,000 species in 30 phyla) lives in or on the water. The greatest number and diversity of animal species are still found in the marine environment; in fact, several groups are exclusively marine, *e.g.*, echinoderms and tunicates, amphineurans, scaphopods, cephalopod molluscs, brachiopods, and gephyrean worms (1).

Of these marine organisms, thousands are known or thought to contain biotoxic substances and only less than 1% have been examined for biological activity. Of these, less than six have been evaluated to the point of determining their chemical and pharmacological characteristics (4).

The ocean is an enormous reservoir of all the metabolites elaborated into it by its inhabitants. Analysis of seawater has yielded organic acids, sterols, carotenoids, carbohydrates, proteins, fats, peptides, amino acids, free enzymes, and many other materials. The concentration of these varies from place to place in the seas and determines the resident flora and fauna. Seawater itself has long been known to have antibiotic properties (20).

The importance of some of these widely-scattered and free-floating metabolites cannot be underestimated. For example, some external metabolites dissolved in the ocean waters act or serve as hormones. For these, the terms pheromone and ectohormone are used. Substances of this type elaborated by one marine organism may be assimilated by another and trigger certain behavioral or developmental processes. An example is seen in the progressive development in *Achyla* (Phycomycete), a common nonseptate water mold. The activity of at least seven distinct diffusible substances in the external surrounding environment causes the development of sexuality in this mold.

Bonellia viridus (Echiuroidea), a marine worm, elaborates an ectohormone which determines the sex of its ciliated larvae which carry the potentialities of both sexes. The masculinizing substance here is known as bonellin (2, 20). The effectiveness of some of these substances at great dilutions is amazing. Consider the diffusible substance, fertilizin, elaborated by certain sea urchins and echinoderms. This bluish-red pigment is capable of attracting sperms to eggs in dilutions up to one part in 2,500,000,000 parts of water!

It is little wonder then, that fungicidal, growth-inhibiting, antitumor, antiviral, antibiotic, hemolytic, analgesic, cardioinhibitory, and other active substances have been found in marine organisms and their surrounding environment.

The existence of a food chain in the sea where phytoplankton (dinoflagellates, diatoms, *etc.*) convert water and dissolved carbon dioxide (from bacterial activity) *via* sunlight into carbohydrates, fats, *etc.*, which feed the grazers of the sea (zooplankton, *viz.*, crustaceans, larval invertebrates, *etc.*) which feed on still larger fish, points up the important interactions, interdependences, and the variety of biogenetic capabilities of various organisms.

It is not difficult then to see why or how certain toxic dinoflagellates can affect the entire food web by passing along their poisons which may be concentrated in various organs of different organisms anywhere throughout the food chain. Marine algae have phenomenal ability to concentrate and retain chemical substances from the marine environment. Certain seaweeds still serve as good sources of iodine.

It is estimated that more than 30,000 intoxications occur each year from eating poisonous marine products. Less than 20% of the cases are properly diagnosed as to the exact etiological agent (4). It may be due to toxins passed through the food chain or to unknown pathogenic strains of marine bacteria or other unknown factors. Intensive ecological researh on the food web of ichthyotoxic fish is certainly indicated here.

CURRENT STATUS OF MARINE PHARMACEUTICALS

There are many reasons why the successful development of marine pharmaceuticals continues to be a difficult problem. There is a lack of trained personnel; a lack of a multidisciplinary approach; enormous procurement problems, especially for sufficient quantities of material to carry out thorough and complete studies; problems in the culture of marine organisms in the laboratory for study; problems in the screening of crude extracts for biological activity; difficulties inherent in tedious natural product extraction separation, and characterization methodology; and problems concerned with adequate patent protection for products developed. Once a drug is found efficacious for a particular disorder, a pharmaceutical company follows through with its marketing. This may take up to seven years.

A new drug application for the FDA can cost up to seven million dollars, this chiefly to develop all the information required. Then the FDA must clear the item for safety and efficacy before it can be marketed. The answer to some of these difficulties lies in the successful pooling of ideas, talent, and experience of several related overlapping disciplinary areas, *e.g.*, ecology, venomology, taxonomy, ethnobotany, pharmaceutical chemistry, pharmacognosy, pathology, oceanography, *etc.* (18), and concentrating efforts on certain problems which are most likely to yield new useful and marketable pharmaceuticals.

Even with all these obstacles, many classic drugs continue to be obtained from marine sources. A few include agar from species of *Gelidium*, *Gracilaria*, and *Hypnea;* cod liver oil and sodium morrhuate from the codfish, *Gadus morrhua;* protamine sulfate from the sperm or mature testes of salmon; the alginates from species of *Fucus;* carrageenan from *Chondrus;* spermaceti from the sperm whale; ichthammol from bituminous schists containing fossil fish; and many others (21).

Today, contemporary experimental marine biology has given us a brief hint of many other newer pharmacologically active substances from marine organisms. Some are usually categorized or considered as toxins or poisons. However, their isolation, characterization, and ultimate attenuation should lead to useful medications. If this is not possible directly, another route may be found in the synthesis of compounds analogous to the pharmacologically active molecule from nature.

TAXONOMIC SURVEY OF MARINE ORGANISMS YIELDING PHARMACEUTICALLY INTERESTING COMPOUNDS

Because of the vast body of information available, and the lack of a broad chemical or pharmacological base on which to categorize this information, the marine organisms of biomedical interest are discussed by taxonomic groupings. Table I is an attempt to combine a taxonomic and pharmacological method of summarizing some of this information for quick reference. A few examples from each phyla are given in order to see the broad distribution of potential drug leads in practically all categories of marine organisms.

Table I—Types of Pharmacological Activity and Potential Drugs from Marine Organisms

Taxonomic Groups (Phyla)	Type of Pharmacological Activity Observed in Various Organisms[h]								Other Activity	Nature of Toxin and Toxicity	Potential Pharmacological-Drug Use	Ref.
	CNS[a]	RS[b]	NMS[c]	ANS[d]	CVS[e]	GI[f]	Local[g]	Antibiotic Activity				
Monerans												
Schizophyta												
Marine bacteria												
Bacillus spp.	—	—	—	—	—	—	—	+		Antibacterial principles	Potential source of antibiotics	(42–53)
Micrococcus spp.	—	—	—	—	—	—	—	+				
Chromobacterium spp.	—	—	—	—	—	—	—	+				
Aeromonas spp.	—	—	—	—	—	—	—	+		Antibacterial, antifungal, and antiyeast principles	Antibiotics with control against yeast and fungal pathogens	
Pseudomonas spp.	—	—	—	—	—	—	—	+	Antifungal and antiyeast activity			
Vibrio spp.	—	—	—	—	—	—	—	+				
Flavobacterium spp.	—	—	—	—	—	—	—	+				
Alcaligenes spp.	—	—	—	—	—	—	—	+				
Flavobacterium piscicida	+	—	—	—	—	—	—	+		Toxin	CNS drug	
Marine Actinomycetes												
Nocardia spp.	—	—	—	—	—	—	—	+		Antibacterial	Antibiotic	
Cyanophyta (blue-green algae)												
Lyngbya majuscula	—	—	—	—	—	—	+	+	Toxic	Antibacterial	Antibiotic	(20, 24, 54–64)
Microcystis aeruginosa (fresh-water)	+	—	—	—	—	—	—	—	Toxic	Polypeptide endotoxin	CNS drug	
Anabaena flos-aquae (fresh-water)	+	—	—	—	—	—	—	—	Toxic	Polypeptide	CNS drug	
Phormidium spp.	—	—	—	—	—	—	+	—	Stimulates growth of bacteria, plant, animal, algae cultures	Growth stimulant	Wound healing	
Nostoc rivulare	—	—	—	—	—	—	+	—	Carcinogenic	Unknown	Study of cancers	
Protistans												
Rhodophyta (red algae)												
Digenea simplex	—	—	—	—	—	—	+	—	Antihelminthic	Kainic acid	Against parasitic intestinal worms (ascaris)	(8, 24, 60, 65–108)
Chondrus crispus	—	—	—	—	—	—	—	—	Antiviral	Carrageenan	Antiviral drug	
Gelidium cartilagenium	—	—	—	—	—	—	—	—	Antiviral	Polysaccharide	Antiviral drug	
Phaeophyta (brown algae)												
Rhodomela larix	—	—	—	—	—	—	—	+		Brominated phenolic compound	Antibiotic	
Laminaria spp.	—	—	—	—	—	—	—	—	Blood anticoagulant	Laminarin	Anticoagulant	
Chlorophyta (green algae)												
Chlorella spp.	—	+	+	—	—	+	—	+	Toxic	Unknown, oxidation products of fatty acids	NMS studies, antibiotics	
Chlamydomonas reinhardtii	—	—	—	—	—	—	—	+		Fatty acids	Antibiotic	
Chrysophyta (diatoms)												
Ochromonas spp.	+	—	+	—	—	—	—	—	Ichthyotoxic	Unknown	CNS and neuromuscular drugs	

(*Continued on next page*)

Table I—(*Continued*)

Taxonomic Groups (Phyla)	Type of Pharmacological Activity Observed in Various Organisms[h]								Other Activity	Nature of Toxin and Toxicity	Potential Pharmacological-Drug Use	Ref.
	CNS[a]	RS[b]	NMS[c]	ANS[d]	CVS[e]	GI[f]	Local[g]	Antibiotic Activity				
Prymnesium parvum	+	—	+	—	—	—	—	—	Ichthyotoxic, hemolytic, cytolytic, antispasmodic activities	Prymnesin	CNS and neuromuscular drugs	(127–131)
Phaeocystis pouchetii	—	—	—	—	—	+	—	+		Acrylic acid	Broad spectrum antibiotic for GI tract	
Pyrrophyta (dinoflagellates)												
Gymnodinium spp.	+	+	+	+	+	+	+	—		Alkylguanidine compounds	CNS drugs	(109–125)
Gonyaulax spp.	+	+	+	+	+	+	+	—		Alkylguanidine compounds	CNS drugs	
Invertebrates												
Porifera (sponges)												
Tedania toxicalis	—	—	—	—	—	—	+	+		Unknown	Antibiotic	(186–196)
Suberites domunculus	—	+	—	—	+	+	+	—		Unknown	?	
Microciona prolifera	—	—	—	—	—	—	—	+		Ectyonin	Antibiotic	
Haliclona variabilis	—	—	—	—	—	—	—	—	Aggregation factor	Protein	Study of healing process	
Cryptotethya crypta	—	—	—	—	—	—	—	—	Growth regulators	Nucleosides, spongothymidine, and spongouridine	Antagonists in nucleic acid metabolism	
Coelenterata (*cnidaria*)												
Hydroids												
Physalia physalis	+	+	+	+	+	+	+	—		5-HT, low-molecular weight proteins and peptides	Cardioactive and neuromuscular drugs	(203–230)
Jellyfish												
Chironex fleckeri	+	+	+	+	+	+	+	+		5-HT, low-mol. wt. proteins and peptides	Cardioactive and neuromuscular drugs	
Aurelia aurita	—	—	—	—	—	—	—	—	Neurohumoral compounds	Extract affects neurofunction of related species	CNS drug	
Sea Anemones												
Actinea equina	+	—	+	—	—	+	+	—		Tetramine, homarine	Cardioactive and neuromuscular drug	
Rhodactis howesii	+	—	—	—	—	—	—	—	Anticoagulant	—	CNS drug, anticoagulant	
Corals												
Acropora palmata	+	—	—	—	—	—	+	—		Unknown	?	
Plexaura crassa	—	—	—	—	—	—	—	+	Toxic	Crassin	Antibiotic	
Echinodermata												
Starfish												
Asterias spp.	—	—	—	—	+	—	—	—	Hemolysis, toxic, sperm immobilization, induces egg and sperm shedding	Autotomizing toxin, saponins, asterotoxin	Tissue regeneration studies, sperm inactivation studies, egg maturation studies	(232–280)
Sea urchins												
Paracentrotus lividus	+	+	+	+	—	—	+	—	Toxin in ovaries and spines	Unknown	CNS drug, NMS drug	
Tripneustes gratilla	+	+	+	+	+	—	+	—	Toxin in ovaries and spines	Toxic protein (acetylcholine-like)	Neuromuscular blocking agents	
Sea cucumbers												
Actinopyga agassizi	—	—	+	—	—	—	+	—	Hemolytic, toxic, cytotoxic, antitumor activity	Holothurin (complex of steroidal glycosides, salts and polypeptides)	Neuroactive drug	
Mollusca												
Gastropods												
Haliotis spp.	—	—	—	—	—	—	—	+	Antiviral	Paolin I is antimicrobial and paolin II is antiviral; both are protein	Antiviral drugs	
Conus spp.	+	+	+	+	+	—	+	—	Toxic	Venom contains mixture of peptides and ammonium Compds. (homarine, *N*-methyl-*N*-methylpyridinium, γ-butyrobetaine) with protein	Neuromuscular and CNS drugs	
Neptunea arthritica	—	+	+	—	+	—	—	—		Saliva toxin (tetramine)	Neuromuscular and CNS drugs	
Murex spp.	+	+	+	+	+	—	—	—		Murexine	Neuromuscular and CNS drugs	

(*Continued on next page*)

Table I—(*Continued*)

Taxonomic Groups (Phyla)	Type of Pharmacological Activity Observed in Various Organisms[h]								Other Activity	Nature of Toxin and Toxicity	Potential Pharmacological-Drug Use	Ref.
	CNS[a]	RS[b]	NMS[c]	ANS[d]	CVS[e]	GI[f]	Local[g]	Antibiotic Activity				
Bivalves												
Mytilus spp.	—	+	+	+	+	+	+	—		Poisoning caused by ingestion of toxic dinoflagellates	Neuroactive drugs	
Mercenaria mercenaria	—	—	—	—	—	—	—	—	Growth inhibitor	Mercenene	Antitumor drug	
Octopuses												
Octopus spp.	+	+	+	+	+	+	+	—	Hemolysis	Toxic venom in saliva; tyramine, octopamine, 5-HT, histamine, protein (cephalotoxin)	CNS drugs	
Annelida (segmented worms)												
Lumbriconereis heteropoda	—	—	+	+	+	+	+	—	Anesthetic for insects	Nereistoxin	Neuroactive drug	(317–326)
Anthropoda (joint-footed marine animals)												
Carcinoscorpius notundicauda	+	—	+	—	+	+	+	—		Unknown	Neuromuscular drugs	(331–338)
Carcinus maenas	—	—	—	—	+	—	—	—	Increase	6-HT, a mucopeptide	Cardiac drug	
Vertebrates												
Fish												
Agnatha (jawless fish)												
Eptatretus stoutii	—	—	—	—	+	—	—	—		Eptatretin (low-mol. wt. amine) obtained from heart	Cardioactive agent (hypertensive)	(339–340)
Chondrichthyes (cartilaginous fish)												
Sharks												
Somniosus microcephalus	+	—	+	—	—	+	—	—	Visual disturbances	Form of ciguatera toxin (?) due to ingestion of livers and musculature	CNS drug	(3, 341)
Hexanchus grisseus	+	—	+	—	—	+	—	—	Visual disturbances			
Stingrays												
Dasyatis pastinacus	+	+	—	+	+	+	+	—	Toxic	Sting venom is protein in nature	CNS or cardioactive drugs	(342–383)
Urobatis halleri	+	+	—	+	+	+	+	—	Toxic			
Osteichthyes (true or bony fish)												
A. Ichthyosarcotoxic (poison in musculature, viscera, skin)												
1. Ciguatera group (fish poisoning characterized by GI and neurological effects). Includes over 300 species in 12 families, *e.g.*, sturgeon fish, sea basses, snappers, barracudas, *etc.*	+	+	+	+	+	+	+	—		Herbivorous spp. may feed on toxic blue-green algae (cyanophyta). Carnivorous spp. feed on toxic herbivorous spp. and accumulate and concentrate the toxin(s). Toxin probably a mixture. Anticholinesterase implicated.	Neuroactive or gastrointestinal drugs	
2. Tetraodon group (puffer or fugu poisoning of neurotoxic type)												
Diodontidae (porcupine fish, 10 spp.)	+	+	+	+	+	+	+	—	Toxic	Toxin most toxic of ichthyosarcotoxic types.	Neuroactive drug	
Molidae (sunfish, 1 spp.)	+	+	+	+	+	+	+	—	Toxic	Toxin concentrated in ovaries or testes, liver and intestines. Musculature free of poison.		
Tetraodontidae (puffers, 40 spp.)	+	+	+	+	+	+	+	—	Toxic	Toxin known as tetrodotoxin. ($C_{11}H_{17}N_3O_8$)		
3. Scombroid group (mackerel-like fish, tunas, skipjacks, and bonitos)	+	—	—	—	—	+	+	—		Toxin called "saurine." Forms if fish are inadequately preserved. Has histamine-like properties.	?	

(*Continued on next page*)

Table I—(*Continued*)

Taxonomic Groups (Phyla)	Type of Pharmacological Activity Observed in Various Organisms[h]								Other Activity	Nature of Toxin and Toxicity	Potential Pharmacological-Drug Use	Ref.
	CNS[a]	RS[b]	NMS[c]	ANS[d]	CVS[e]	GI[f]	Local[g]	Antibiotic Activity				
4. Clupeoid group (herring-like fish of tropical Pacific)	—	—	—	—	—	—	—	—		Toxin produces symptoms somewhat like ciguatera poisoning	?	
5. Hallucinogenic group [mullet and surmullet (goatfish)]												
Mugil cephalus	+	—	+	—	—	+	+	—	Light-headedness	Unknown	CNS drugs	
Neomyxus chaptalli	+	—	+	—	—	—	+	—	Hallucinations			
Paraupenus chryserydros	+	—	+	—	—	—	+	—	Depression			
Upeneus arge	+	—	+	—	—	—	+	—	Violent nightmares.			
B. Ichthyootoxic fish (poison in gonads)												
Scorpaenichthys marmoratus	+	—	—	—	—	+	—	—		Unknown	?	
C. Ichthyohemotoxic fish (poison in blood)												
Muraena helena	+	—	+	—	—	+	—	—		Toxic protein (?) in ingested blood	?	
D. Venomous fish												
1. Weeverfish												
Trachinus draco	+	—	—	+	+	—	+	—		Envenomation on mishandling venomous spines. Venom contains protein, 5-HT, histamine releaser	Cardiovascular drugs	
Trachinus vipera	+	—	—	+	+	—	+	—				
2. Scorpion fish (rock fish)												
a. Zebrafish												
Pterois spp.	+	+	+	—	+	—	+	—		Venomous spines	Cardioactive drugs	
Dendrochirus spp.	+	+	+	—	+	—	+	—		Venom protein in nature		
b. Scorpaena												
Scorpaena guttata	+	—	—	—	+	+	+	—		Venomous spines	Cardioactive drugs	
Urolophus halleri	+	—	—	—	—	—	+	—		Venom protein in nature		
c. Stone fish												
Synanceja horrida	+	+	+	—	+	—	+	—		Venom protein in nature	Cardioactive, muscle-relaxing drugs	
Amphibians												
Salamandridae (true newts)												
Taricha torosa	+	+	+	+	+	+	+	—	Toxic	Toxin concentrated in skin, muscles, and blood. Toxin identified with tetrodotoxin	Neuroactive drug	(352, 384–388)
Reptiles												
Marine turtles												(3, 13, 395–400)
Chelonia mydas	+	—	—	—	—	+	+	—		Toxic on ingestion	?	
Eretmochelys imbricata	+	—	—	—	—	+	+	—		Toxin unknown		
Dermochelys corialea	+	—	—	—	—	+	+	—				
Sea snakes												
Pelamis platunas	+	+	+	+	—	—	+	—		Venomous fangs	Neuroactive, CNS	
Enhydrina schistosa	+	+	+	+	—	—	+	—				
Hydrophis caerulescens	+	+	+	+	—	—	+	—				

[a] CNS = Central Nervous System (nausea, headache, confusion, visual disturbances, nervousness, drowsiness, *etc.*). [b] RS = Respiratory System (depression, distress, syncope, dyspnea, *etc.*). [c] NMS = Neuromuscular System (muscle weakness, incoordination, spasms, curare-like action, paralysis, *etc.*). [d] ANS = Autonomic Nervous System (pupil dilation, anticholinesterase activity, parasympathetic action, *etc.*). [e] CVS = Cardiovascular System (cardiac stimulation, bradycardia, congestion, myocardial ischemia, *etc.*). [f] GI = Gastrointestinal (vomiting, diarrhea, abdominal pain, *etc.*). [g] Local = pruritis, parasthesias, pain, necrosis, edema, *etc.* [h] + = activity; — = no activity.

PLANTS OF THE SEA

Monerans

Although disputed (40), the existence of specific marine bacteria is well established and of the approximate number of living species (*ca.* 1,500), some 12% are ubiquitous marine forms. They show enormous range in habitability and are the major liberators of mineral nutrients (particularly dissolved carbonates) for plants, and carry out decomposition of expired marine organisms.

About 95% of the bacteria are Gram-negative rods, and are active flagellated forms. Many are also sedentary in habit and attach themselves tenaciously to solid surfaces *via* mucilaginous holdfasts. Nearly 70% are pigment producers (orange, yellow, brown, pink, green) and many show fluorescence.

The largest populations are found near the shore

(50,000–400,000 bacteria/ml. of seawater) while in the open sea the population is low (40 bacteria/ml. of seawater). Up to 160,000 viable bacteria/ml. have been found on the sea-floor muds in the West Indies. Geological cores taken 5 m. below the surface of the ocean floor have even yielded bacteria (41).

Sea water also contains bacteriophages, *i.e.*, bacterial viruses. The source of many of the free enzymes found in seawater, particularly concentrated on the sea bottom, is considered to be bacteria and other microorganisms. Some of these enzymes continue to function long after the organisms responsible for their production have disappeared. The enzymes rise to the surface during upswellings and if seitz-filtered seawater, free of bacteria and other microorganisms, is analyzed, oxidases, reductases, and other enzymes capable of catalyzing changes in phosphates, oxygen, ammonia, nitrates, *etc.*, will be found (20).

Reference to Table I shows that this group is prominent, like their terrestrial counterparts, in showing antibiotic activity. Rosenfeld (42) recorded the antibacterial activity of almost 60 marine microorganisms and found that six (*Bacillus* and *Micrococcus* species) were effective against several nonmarine microorganisms.

Grein *et al.* (43) found 70 active isolates of actinomycetes of some 166 derived from littoral sediments and materials suspended in seawater. These were effective against both Gram-positive and Gram-negative bacteria.

In a study of antibiotic properties of microorganisms isolated from various depths (0–3,500 m.) of the world oceans, Krasil'nikova (44) found 124 active microbial isolates out of 326 collected. Most (*ca.* 217) were nonspore-forming bacteria, at least 79 were cocci, and 21 were spore forming. Eight yeasts and one actinomycete were also obtained. This latter actinomycete showed a large antibacterial spectrum. The antibiosis was shown against the test organisms, *S. aureus*, *E. coli*, *Mycobacterium luteum*, and *S. cerevisiae*.

In a series of papers by Buck *et al.* (45–47) more antibacterial and antifungal activity was shown (seven active out of 132 isolates) in addition to antiyeast activity. The antiyeast activity was found in an isolate of a marine Pseudomonad. The test organisms were a number of terrestrial, marine, and human yeasts. The possibilities of developing agents for treatment of yeasts pathogenic to man may be afforded by continued studies of this type.

A fungus, *Cephalosporium acremonium*, claimed as a "Healer from the Sea" (48), was isolated from a sewage outlet off the coast of Sardinia. This organism is the source of cephalothin, a semisynthetic derivative of cephalosporin C, an antibiotic with action similar to that of benzyl penicillin but insensitive to penicillinase and therefore active against a number of penicillin-resistant *Staphylococci* and some Gram-negative species of bacteria. Cephalothin[2] is widely used in medicine today (35, 49, 50).

The subject of bacterial toxins is still important, particularly with regard to the ecological balances in the marine environment. Bein (51) has described a new species of bacteria (*Flavobacterium piscicida* Bein) which may have been implicated in the mass mortality of fish on the southwest coast of Florida. An abnormal bloom or "red tide" occurred here in 1951. Later chemical studies by Meyers in collaboration with Baslow *et al.* (52) indicate the toxic substance to be a fairly stable small volatile molecular species which causes deleterious effects on the nervous system of fish.

The subject of antibiosis of marine microorganisms was considered in a recent symposium (53). All of the leads, discussed above point to potential antibiotic, antifungal, antiyeast, and central nervous system-active agents.

In the phylum Cyanophyta (blue-green algae) of the moneran kingdom several marine and fresh-water genera show interesting pharmacological activity. *Lyngbya majuscula* has been implicated in outbreaks of dermatitis among swimmers (54–56), and toxicity in fish and mice (57). However, *Lyngbya majuscula* has also shown antimicrobial, antiviral, fungicidal, and other types of growth-inhibitory properties in preliminary pharmacological studies (4).

Toxic cyclic polypeptides capable of producing quick death in laboratory animals have been isolated from *Microcystis aeruginosa* and *Anabaena flos-aquae* (58). The LD_{50} for mice, of the purified peptide from *M. aeruginosa* is 0.5 mg./kg. The MLD for mice of a 95% ethanol extract of dried *Anabaena flos-aquae* is 40–320 mg./kg. The toxin of the former organism affects the liver and the central nervous system while the toxin of the latter affects only the nervous system (59). These two organisms, in addition to *Aphanizomenon* seem to be the primary causative agents in many dramatic and serious poisonings in livestock. Gorham (58) reports that some animals, even as large as a mature cow, have died in less than 30 min. after ingesting these algae.

Schwimmer and Schwimmer (24) give 235 references to the toxic properties of various marine and fresh-water algae. Gastrointestinal, hepatic, neuromuscular, respiratory, and cardiovascular effects of poisoning by several genera on a variety of animals and man are given. Human mycoses and tumor formations associated with algae are also discussed. In the latter, the use of *Nostoc rivulare* to produce tumors experimentally may be helpful in elucidating the mechanism of cancer induction.

Antibacterial substances produced by marine algae are given by Sieburth (60) and other important properties are reported by Lewin (61) and Jackson (62).

The growth-stimulating properties of algal extracts on bacteria, plant and animal tissue cultures, and other algae, had been noted early by Feller (63). In a continued and much later research effort concerning stimulating substances produced by certain algae, Lefevre (64) concluded that fresh-water algae (*Phormidium* spp.), can be used in therapeutics. Only growth-stimulant and no antibiotic properties were detected. Over 40 clinical assays on humans and animals showed positive results, characterized as spectacular, in the treatment of infected wounds, dermal ulcers, and scar healing.

As an example of how an ecological study can turn up interesting biomedically useful facts, Sieburth (20, 77), in studying why Antarctic penguins had a sterile intestinal tract, found that they fed on the ubiquitous Krill

[2] Keflin, Eli Lilly & Co., Indianapolis, Ind.

(crustacean) which in turn fed on the blue-green algae, *Phaeocystis pouchetti*, which elaborates acrylic acid. Acrylic acid is a strongly active and effective antibiotic against a number of pathogenic organisms, including bacteria and yeasts.

It is obvious that these results beg for further research in phycology and phycotherapy.

Protistans

In the protistans, which include the red, brown, and green algae, the diatoms and dinoflagellates, there are many members possessing a wide variety of pharmacological activity. Generally, certain genera of the red, brown, and green algae contain useful phycocolloids, are good sources of thiamine, niacin, riboflavin, folic acid, alpha tocopherol, vitamin A, ascorbic acid, and ergosterol, and have a relatively high mineral content (up to 38.9%), *e.g.*, halides, sulfates, phosphates, and oxides of calcium, magnesium, potassium, sodium, and trace elements (18).

The phycocolloids continue to be used extensively in the food, drug, textile, and cosmetic industries because of the gelling, emulsifying, thickening, suspending, and sizing properties which they possess.

The silicaceous diatoms have traditionally been widely used as filtering aids, scouring powders, and adsorbants while the dinoflagellates continue to serve as important nutritional sources in the food chain of the sea. However, certain species of dinoflagellates are toxic.

Because the phytoplanktonic organisms and higher algae are the primary producers in the marine environment, they elaborate many materials into it. Of interest here, besides what has been mentioned above, are the elaborated principles which have broad-spectrum antibiotic activity. The types of compounds thus far characterized as antibacterials, are varying molecular weight fatty acids, derivatives of certain terpenes and chlorophyll, brominated phenolic compounds, acrylic acid, and certain sulfated polysaccharides (2, 4, 8, 24, 60, 63, 65–88).

Most of these studies have been carried out by teams of one or two researchers and have been limited to the detection of antibiosis by zones of inhibition using standard agar cultures of test organisms. Relatively few investigations have been carried to the point where the antibiotic principles were identified, probably due to limited amounts of materials and work necessary. Thus the time is long overdue for large-scale collection of those showing activity and research efforts directed toward growing these in pilot plant operations in order to facilitate isolation of new potential antibiotics. By use of the fermentation techniques commonly used in industry, this could be achieved possibly yielding new antibiotics which may prove useful in combating resistant strains of common pathogenic organisms and perhaps even destroying those which have thus far been unaffected by all known chemotherapeutic agents. If any of this research has been carried out by any of the pharmaceutical concerns, little has appeared in the scientific literature about it.

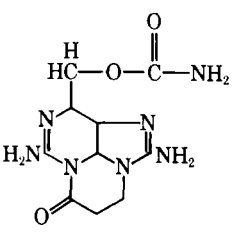
In addition to the references to antibiotic substances elaborated by algae and given above, there are many others which refer to additional pharmacological activity of other algae, diatoms, and dinoflagellates (89–108).

Among those of interest here are a bacterial-toxin type of phospholipase (lecithinase C) in a marine phytoplanktonic chrysomonad (89); *Gonyaulax catenella* (dinoflagellate) toxin (90); *Caulerpa* (green algae) toxin (69); several marine algae toxins (92); vitamins in marine algae (96); algal inhibition by *Fucus vesiculosis* (brown algae) extracts (97); toxicity of *Prymnesium parvum* (dinoflagellate) (98); induced shellfish poisoning in chicks with *Gymnodinium breve* (100); electrophoretic separation and analysis of serum proteins, hemoglobin, lipoproteins, and isoenzymes using agarose from seaweed (101); fishtoxins in *Ochromonas* (diatom) (102); hypocholesterolemic agents derived from sterols of marine algae (103); anticoliform activity of seawater associated with the termination of *Skeletonema costatum* blooms (104); antibacterial and antiviral activities of algal extracts (106); toxicity of algae (107); and antiviral kelp (brown seaweed) extracts (108).

In the phylum Pyrrophyta, most toxicities appear with members of the dinoflagellates. The dinoflagellates contain nearly 1,000 species, many being components of plankton. At least 22 species are implicated in poisonings (4). During certain times, weather disturbances and other factors cause the overgrowth of these organisms to the point where there is considerable discoloration of the water to produce what is known as "red tide." The excessive accumulation of the dinoflagellates (blooms) often produces a mass mortality of many fish and other organisms in the surrounding environment. While many of the factors responsible for these toxicities may be physical in nature (oxygen depletion in water, physical asphyxiation, *etc.*) there is a possibility that certain toxins are implicated. It is known for example, that toxic dinoflagellates are the cause of paralytic shellfish poisoning.

Chemical and pharmacological studies have revealed that this toxin (also referred to as *Gonyaulax* toxin, saxitoxin, mussel poison, or mytilotoxin) appears to be a single chemical entity, or at least consisting of closely related structures, and is among the most toxic materials known to man. The equivalent of 1 mg. of purified toxin on ingestion has caused death in man.

Nigrelli (2) and Russell (3) have recently summarized the current knowledge of this toxin. Essentially, the alkylguanidine group is common in some of the suggested formulas (4) and a molecular formula of $C_{10}H_{17}N_7O_4 \cdot 2HCl$ with a molecular weight of 372 has been assigned to it (3, 4, 23, 109–116). Gonyaulax poison (I)



I

shows a substituted purine which is another proposed structure of the toxin (3).

Pharmacological studies have revealed that this is a

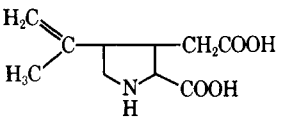
potent neurotoxin having dramatic central and peripheral effects (112, 117–124). The central effects include strong action on the cardiovascular and respiratory centers, and the peripheral effects include action on the neuromuscular junction and sensory nerve endings. Experiments with isolated hearts show that the toxin has direct effect on the heart, and this is believed to be the cause of the cardiovascular crisis and subsequent precipitous deleterious changes in the systemic arterial blood pressure.

Recent studies further show that by specifically preventing an increase in ionic permeability usually associated with sodium influx, the toxin blocks action potentials in nerves and muscles. It does this without changing chloride or potassium conductances (125, 126).

To summarize, this toxin produced by *Gonyaulax catanella* is one of the most potent and pharmacologically active substances yet isolated from marine organisms. It is active in nanomolar concentrations and is about 100,000 times more active than the known conventional local anesthetics (procaine, cocaine) (125). The molecular structure of the toxin (I), thus far thought to be a substituted purine compound with certain unidentified side chains, certainly offers a model on which the synthesis of new and potent cardioactive, central nervous system active, or local anesthetics may be based.

Another interesting toxic factor from the fresh-water chrysomonad (dinoflagellate), *Prymnesium parvum*, is prymnesin. Although still uncharacterized and believed to be a mixture, it has shown hemolytic, ichthyotoxic, antispasmodic, and cytolytic effects on various animals (98, 127–131).

The red algae, *Digenea simplex*, long used in Japanese folk medicine as an anthelmintic, has yielded kainic acid (2-carboxy-3-carboxymethyl-4-isopropenyl-pyrrolidine) (II). Kainic acid was formerly known as digenic acid.

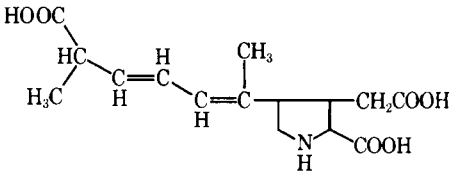


II

Kainic acid is the active principle in the algae and is widely used in Japan for its vermifuge or anthelmintic properties against the parasitic round worm, *Ascaris lumbricoides*, the whip worm, *Trichuris trichura*, and the tape worm *Taenia* spp.

Kainic acid has few side effects and has produced no pathological changes in the digestive tract when administered to mice. It apparently acts by dissolution and separation of the intestinal epithelium of the round worm (*Ascaris lumbricoides*) in addition to causing mucoid degeneration of its epithelial cells. It also causes motor paralysis and inhibition of the action of dehydrogenase in the muscles so that tissue respiration is depressed. Kainic acid in combination with santonin is available in powder and tablet form. Digesan syrup used in the treatment of ascariasis, trichuriasis, and oxyuriasis, is also available and contains these same ingredients in combination with piperazine adipate. The Takeda Pharmaceutical Industries, Ltd., Osaka, Japan, produces these products; however, they are not generally available in the United States because of certain FDA regulations (21, 132–141).

Domoic acid [2-carboxy-4-(1-methyl-5-carboxy-*trans*-*trans*-*S*-*trans*-1,3-hexadienyl)-3-pyrrolidinacetic acid] (III), obtained from *Chondria armata* (red algae) is currently being investigated for its potent exterminating effects on *Oxyuris* as well as *Ascaris* worms (138, 142–148). These are good examples of where folklore use has led to useful drugs.



III

It was observed quite early that agar, when used as a substrate for certain types of virus-infected tissue (*e.g.*, EMC, or encephalomyocarditis virus), possessed the ability to inhibit the development of the virus (2). Subsequent investigations revealed that the active principle was a sulfated polysaccharide.

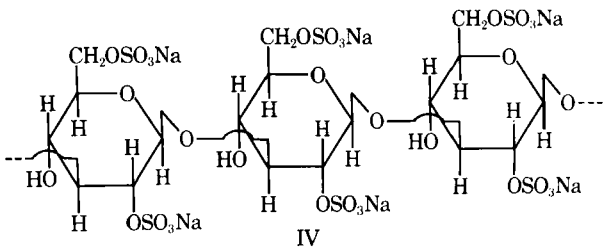
Gelidium cartilagenium, a common source of agar, yielded an active component which was found to be a linear polysaccharide of D-galactose linked 1 to 3. Ethereal sulfate groups were found in low number through the polymer.

In the case of *Chondrus crispus*, the active compound was identified as carrageenan, also a linear polysaccharide consisting of D-galactose units linked 1 to 3. Here, some L-galactose, a ketose, and ester sulfate moieties were also found.

The antiviral properties of both have been attributed to their galactan units since other similar polysaccharides lack this and have no antiviral activity. The specific antiviral activity was shown against influenza B and mumps virus in embryonated chicken eggs, even after 24-hr. inoculation. Protection on the order of 70% was also afforded mice given intranasal viral infections (PVM) (149).

Takemoto (150) tested a number of natural and synthetic polyanionic substances and the sulfated polysaccharides mentioned above and found that they could adversely affect the growth of a large number of animal viruses in tissue cultures. These included herpes viruses, picornaviruses, arboviruses, and myxoviruses (2, 150).

Another seaweed, *Laminaria coloustoni*, and other species yield the polysaccharide, laminarin (IV shows the sodium salt of laminarin sulfate), which consists



IV

essentially of β-D-glucose residues joined mainly through

1:3-type linkages. The highly sulfated derivatives of laminarin have anticoagulant properties comparable to heparin. In addition, the laminarins with few sulfate groups have antilipemic properties, without anticoagulant activity. This latter property allows the use of lower-sulfated laminarin derivatives as effective antilipemic agents without the hazards of concomitant anticoagulant action. It would appear that assessment of these agents, capable of lowering the amount of fatty substances in the blood against atherosclerosis, should yield fruitful results.

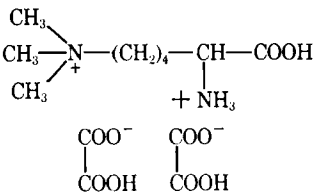
Much biological testing both *in vivo* and *in vitro* has shown that the highly sulfated laminarin derivatives are about one-third as effective as heparin in delaying blood coagulation in dogs and guinea pigs. Single large doses appear to be nontoxic whereas longer dosage regimens may have deleterious effects on the gastrointestinal tract (2, 151–159).

Carrageenan has recently been found to be useful for its antipeptic or antiulcer properties as well as a potentially useful anticoagulant and antithrombic substance. Anderson (160–165) has studied the effects of this algal polyanion on inhibition of peptic activity, on protection against histamine ulceration, and on acidity and volume of histamine-stimulated gastric secretion, using the guinea pig.

Bianchi reports on the antipeptic and antiulcerogenic properties of a synthetic sulfated polysaccharide (166). Hawkins reports on the antithrombic activity of carrageenan in human blood (167), Heineken (168) discusses carrageenan in the management of peptic ulcer, and Houck (169, 170) gives anticoagulant, lipemia clearing, and other effects of anionic polysaccharides extracted from seaweed.

Studies on the antilipemic properties of the algae *Sargassum vulgare* and *Polysiphonia subulifera* are reported by Atkin (171).

Another line of investigation yielded through research on folk medicine led to the isolation of laminine dioxalate [(5-amino-5-carboxypentyl)-trimethyl ammonium dioxalate] (V), a purported hypotensive agent.



V

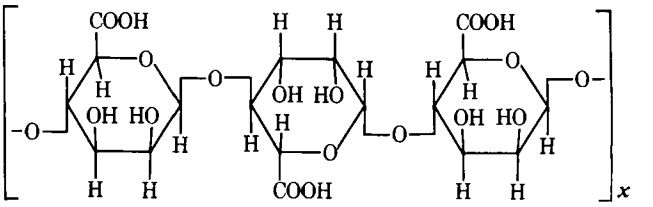
Certain species of *Laminaria* have been used in folk therapy by the laity in the northeastern part of Japan, particularly for prevention and treatment of hypertension. Kameda and Osato (172) reported that *Laminaria* extracts were effective in controlling experimentally induced arteriosclerosis and hypertension in rabbits, fed high doses of cholesterol. In addition' patients with high blood pressure and hypertension who were fed these extracts obtained relief from their symptoms.

Takemoto *et al.* (173–176), following these observations isolated laminine dioxalate and determined the concentration of this principle in 19 different species in the Laminariaceae family. He found that *Laminaria angustata* contained the highest concentration of laminine while others showed a slightly lower concentration, *e.g.*, *L. yezoensis*, *L. cichoriods*, and *Ecklonia cava*.

Heterochordaria abietina, which belongs in the Chordariaceae family, showed large concentrations of laminine. Pharmacological and clinical studies are continuing on the hypotensive agents of these marine algae.

It should be noted that kainic acid, domoic acid, and laminine dioxalate are all new and novel biologically active amino acids obtained from marine algae.

Alginic acid (a long chain of uronic acid groups, joined by 1:4 glycosidic linkages) (VI), and derivatives



VI

obtained from the brown seaweeds (kelp), *e.g.*, species of *Fucus* and *Macrocystis* continue to be useful as sizing material (textiles), in adhesive formulations, as stabilizers and emulsifiers in food products, as cosmetic and pharmaceutical ointment bases, as suspending agents, emulsifying agents, *etc.*

Some of the relatively new uses of the alginates as pharmaceuticals include the use of alginic acid as a tablet-disintegrating agent (3–10% alginic acid in tablets disintegrates them much faster than those containing 15% starch); blood anticoagulants (the sulfuric acid ester of low viscosity algin requires slightly higher doses over heparin, but effects last twice as long); absorbable hemostatic material for control of surface bleeding (mixed sodium-calcium alginate in the form of fine wool or powder has been successfully used in clinical trials and has an advantage of ease of sterilization); in the preparation of sustained-release formulations (propylene glycol alginate and alginate gel slow rate of absorption of drugs administered by intramuscular injection); and in formulations successful as dental impression materials (177–182).

One unique use of sodium alginate is seen in its ability to inhibit uptake of radiostrontium from the human gastrointestinal tract. In human tests, 0.36 μc. of ^{85}Sr was administered orally 20 min. after an oral administration of 10 g. of sodium alginate. Twenty-six days later, 0.48 μc. of ^{85}Sr was given orally. In both phases of the experiment, samples of excreta and blood were collected, and the body retention of ^{85}Sr was measured by means of the Windscale whole body counter.

Based on blood plasma, urine, and body retention measurements, sodium alginate reduced the uptake of radiostrontium from the gastrointestinal tract by a factor of about nine. This discovery is of great importance since ^{90}Sr is probably the most hazardous of all the long-lived fission products occurring in nuclear weapon fallout. The use of sodium alginate appears to be able to remove this contaminant from the body without seriously affecting the availability of Ca, Na, or K

to the body (183, 184). Sodium alginate is available as Kelgin (Kelco Co., Clark, N. J.) and Manucol and Manutex (Alginate Industries Ltd., London, England).

These numerous examples are but a small fraction of the total number of between 10,000–14,000 species of marine plants which yield useful pharmaceuticals and biodynamic principles. Table II shows the kinds of plants in the different plant phyla and divisions which are found in the marine environment (41).

ANIMALS OF THE SEA

Even though there is a wide representation of animals on land, by far the largest number live in the marine environment. Many do not look like animals or even act like them. However, some show primitive digestive systems, other circulate water instead of blood through their bodies, and still others lack anything resembling a head. But because most have multicellular bodies, are generally mobile, and are capable of responding to stimuli, they are grouped with the animals. Their variation is so great that in many cases it is as difficult, if not impossible, to classify them properly as it is to trace their complex lineage from any of the ancestral protistans.

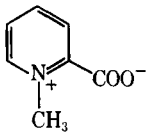
Invertebrates

Porifera (Sponges)—Having neither true organs nor tissues, the sponges are considered the most primitive of all multicellular animals. Food and oxygen are obtained by absorption *via* a filtration process whereby the surrounding water containing these is swept through flagella-lined openings. Sponges vary in size from 0.63 cm. (0.25 in.) to 182.88 cm. (6 ft.), are stationary when mature, and are all marine except for one family which lives in fresh water. Calcareous and siliceous needles constitute their internal skeleton.

Many sponges are considered ageless probably because they are not associated with any of the food cycles. Their resistance to bacterial decomposition is due to certain antibacterial substances which they elaborate into their immediate surroundings. It has also been noted that when the sponge, *Tedania toxicalis*, is placed in a bucket with crabs, fish, and worms, it kills these animals in a short period of time (26).

Relatively few sponges of the more than 5,000 or so species have shown toxicity (2–4, 26). Reference to Table I indicates mostly local pharmacological activity (irritation, pruritus, *etc.*) and some antibiotic activity.

With respect to the local activity, human poisoning may occur through deposition of the toxin(s) in the superficial abrasions produced by the fine, sharp spicules of the sponges (3). Little is known about the nature of the toxins; however, a large number of substances have been isolated and identified by Ackermann *et al.* (185). These include about 11 or so nucleic acids, bases and derivatives, betaine, choline, histamine, guanidine, phosphoarginine, cholesterol, and various derivatives of these and amine compounds like homarine (VII).



VII

Many of these are capable of provoking local and systemic reactions, particularly in lower animals.

Several reports in the literature indicate that sponges

Table II—Proportion of Plants Found in Marine Environment[a]

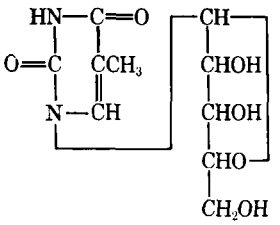
Phylum or Division	Approx. No. Living Species	Proportion, Marine, %	No. of Species, Marine
Monerans			
Schizophyta (bacterial)	1,500	12	180
Cyanophyta (blue-green algae)	7,500 described taxa; probably 200 autonomous species	±75	150 (75% of 200)
Protistans			
Rhodophyta (red algae)	4,000	98	3,920
Phaeophyta (brown algae)	1,500	99.7	1,495
Chlorophyta (green algae)	7,000	13	910
Pyrrophyta (dinoflagellates)	1,100+	93	1,023
Charophyta (stonewarts)	76	13	10
Euglenophyta (euglenoids)	400	3	12
Chrysophyta			
Golden-brown algae	650	±20	130
Coccolithophorids	200	96	192
Diatoms	6,000–10,000	30–50	1,800–5,000
Xanthophyta			
Vaucheria	60	15	9
Mycophyta			
Fungi	75,000	0.4	300
Lichens	16,000	0.1	16
Bryophyta			
Liverworts and mosses	25,000	0	0
Tracheophyta			
Psilopsida, club mosses, horsetails, ferns, cycads, conifers	10,000	0	0
Flowering plants	250,000	0.018 (sea grasses)	45
Total species (marine)			10,192–13,392

[a] Adapted from Dawson (41).

elaborate antibiotic substances (2–4, 26, 186–189). Ectyonin from *Microciona prolifera* is such an antimicrobial agent and it has been shown to inhibit growth of at least six different common bacteria (186). Both the Bahamian sponges *Haliclona viridis* and *Tedania ignis*, also contain antibiotic substances (187).

Nigrelli (2) reports that aqueous and organic solvent-extractable principles from sponges (fresh, dried, and lyophilized) show broad spectrum antibiotic effects particularly against pseudomonas, staphylococci, acid-fast bacteria, and pathogenic yeasts like *Monilia*. He further lists at least 15 different species of sponges showing activity against various pathogenic organisms.

An interesting series of studies has been generated by Bergmann *et al.* (190–194) who isolated and characterized spongothymidine (VIII) and spongouridine (1-β-D-arabinofuranosyl derivatives of thymine and uridine, respectively), from sponges (190–194). These were first



VIII

isolated from the West Indian marine sponge, *Cryptotethya crypta*, which contains them in large concentrations (2). Other unusual nucleic acids have been reported by Nigrelli (195) in *Cryptotethya crypta*.

Cohen (196) brought further attention to these unique nucleic acids and he and others (196–200) report the treatment of certain viral infections and leukemias in man and various laboratory animals with these agents and their derivatives. As Nigrelli (2) has pointed out, these D-arabinofuranosides, have served as models for the synthesis of D-arabinosyl cytosine, an important synthetic antiviral agent. It is capable of inhibiting the growth of sarcoma 180, Ehrlich carcinoma, and L-1210 leukemia in mice.

Another principle of potential value in medicine has been studied in the sponge, *Haliclona variabilis*. It is an aggregation factor which is capable of initiating reassociation in experimentally dissociated cells of the same species. The principle appears to be a protein and it needs calcium for its stabilization. Continued studies should help elucidate healing phenomenon in general and how cells are held together (201).

The first steroid from an animal which was conclusively shown not to be cholesterol was spongosterol from the Mediterranean sponge, *Suberites domuncula* (2, 202).

Coelenterata (Cnidaria)—This phylum includes the hydroids, jellyfish, sea anemones, and corals. They are characterized by having radial symmetry, gut with a single opening, no body cavity, and tentacles with stinging cells. Their habitat is mainly marine with only a few fresh-water species. They range in size from under 2.54 cm. (1 in.) up to 21.3 m. (7 ft.).

This group shows uniform local irritating ability, due mainly to their possession of a stinging apparatus (nematocyst) and an accompanying venom whose potency varies with the different species. The venom is responsible for deleterious systemic effects. The hydroids and jellyfish possess very potent toxins while the sea anemones and corals are of less consequence.

According to Russell (3, 26) and Halstead (4), of the 9,000 or more species of coelenterates, approximately 70 species have been implicated in human toxicities. That more are not involved is probably due to the fact that relatively few have nematocysts capable of penetrating human skin.

The nematocyst or stinging unit is formed within an interstitial cell called the cnidoblast. The cnidoblasts are small rounded or ovoid cells which are widely distributed throughout the epidermis, except on the basal disk of these organisms. They are very abundant on the hanging tentacles and are used offensively and defensively as well as for aids in anchorage. The undischarged nematocyst may be viewed roughly in an analogy, to a rubber ear syringe with a much longer tip which has been pushed into and coiled inside of the syringe bulb. On discharge, the tip is forced out to penetrate the skin like a needle while the venom is ejected from the bulb.

There are many different types of nematocysts each design of which is characteristic of the various species. In fact, their structure is a taxonomic character and at least 17 categories of nematocysts are described (4, 26).

Even though considerable difficulties have been encountered in isolating the venom for pharmacological and chemical studies, Lane *et al.* (3, 203–209) have succeeded in separating undischarged nematocysts from the tentacles of *Physalia physalis* (Portuguese man-of-war) and removing the venom for study. They found that a gallon of tentacle tissue yields about 62–75 g. of nematocysts, and of this, 1 g. of nematocysts contains about 55 million single nematocysts! It is no wonder then how a single contact with even a few tentacles can result in the continuous explosive discharges of thousands of nematocysts each delivering a dose of potent venom.

The nematocysts can retain their potency up to 4 years when stored in the deep freeze. Homogenization of the nematocysts in distilled water, followed by centrifugation, yields a supernatant containing the toxin which is lethal to mice at a dose of 1.7 mg./kg. of body weight. Further experiments have indicated that the toxin is composed of several polypeptides, four of which account for 95% of the pharmacological activity. Generally, the *Physalia* toxin has effects on the conducting system of the mammalian heart, the transmission of the impulse for contraction in crustacean heart, contractibility of rat intestine, and ATPase enzymes from *Cardisoma* gill. These effects have led Lane (209) to postulate that the biological effectiveness of *Physalia* toxin is due to its involvement with membrane transport phenomena.

Investigations by Barnes (210), using an amnion membrane and electrical stimulation to obtain coelenterate venom, have indicated that the lethal dose for *Chironex fleckeri* (sea wasp) is in the range of 0.005 ml./kg. of body weight. Russell (3) has pointed out that this organism is among the most dangerous with respect to

the potency of its venom. In less than 3 min., death can result from its sting!

In addition to the pharmacologically potent proteinaceaous and peptide substances, a number of other active materials have been isolated from the coelenterate venoms (211). These include tetramine (tetramethylammonium hydroxide), a potent paralytic agent (212), serotonin, a pain-producing and histamine-releasing agent, and histamine itself (213, 214).

A number of other quaternary ammonium compounds, *e.g.*, *N*-methylpyridinium hydroxide, trigonelline, homarine, zooanemonin, and γ-butyrobetaine have also been isolated (216).

In a recent review by Picken *et al.* (216) on the chemical constituents of the nematocyst capsule, a list is given of compounds including proteins, hydroxyproline, aspartic acid, alanine, glycine, glutamic acid, sulfur-containing amino acids, tyrosine, proline, uronic acid, hexosamine, aspartic acid, orthodiphenols, a succinoxidase inhibitor, alkaline and acid phosphatases, mineral salts, cholinesterase, and 5-nucleotidase. Certainly some of these also contribute to the overall deleterious effects of the venom.

To summarize, the lethal and paralyzing effects of the toxin are due to several peptides while other severe and local effects are due largely to tetramine, serotonin, histamine and histamine releasers, and some others of the list given above. With respect to the rapid lethal effects, Russell (3) states that this may be due to specific cardiac arrest of either myocardial or central origin (4, 26, 217–220).

Toxicities and other effects have also been observed with the sea anemones, particularly with *Rhodactis howesii* (221–225). An aqueous homogenate of the whole organism has an MLD of 6.2 mg. for a 20-g. mouse. The active principle is considered to be neurotoxic. Accompanying this CNS effect on mice and rabbits, is a systemic hemorrhaging which has been attributed to an anticoagulant fraction. Martin (226) found that the anticoagulant fraction from *Rhodactis howesii* was capable of prolonging the clotting time of human citrated plasma (calcium added to promote coagulation) about 14 times that of the control (heparin).

Even though some of the locally active materials mentioned above have been known from other sources, and their pharmacological properties do not lend themselves to new drug development, the structures of the peptides warrant further study. It is in the arrangement and sequence of the amino acids in these molecules that the potential for new cardioactive and neuromuscular drugs lies. This possibility of a new type of an anticoagulant from *Rhodactis howesii* is obvious.

Other potential pharmaceuticals may be found in the two compounds, crassin from the coral, *Plexaura crassa*, and eunicin from *Eunicea mammosa*. Both possess antibiotic activity and are toxic to *Endamoeba histolytica*, and inhibit the growth of *Clostridium fesseri* and *Staphylococcus aureus*. Crassin acetate is described as an unsaturated polycyclic compound (m.p. 144–145°) and eunicin is thought to be a sesquiterpene lactone ($C_{15}H_{22}O_3$, m.p. 150–152°) (227, 228). It is not clear whether zooxanthellae associated with the corals are responsible for these agents.

Antiyeast activity in a horny coral is described by Buck (229) and the antimicrobial activity of other horny corals is given by Burkholder (230).

It may finally be noted for historical purposes, that the phenomenon of anaphylaxis was discovered during studies on coelenterate toxins (3, 231). Perhaps other important phenomena may be elucidated by continued studies of members in this phylum.

Echinodermata—This phylum includes the starfish, sea urchins, and sea cucumbers. All are characterized by radial symmetry, an internal skeleton with spines often protruding through the skin, and a gut with two openings. Their sizes range from 1.2 cm. (0.5 in.) (sea star) up to 91.44 cm. (1 yard) long (sea cucumber). They are exclusively marine and for the most part are bottom dwellers.

Some 6,000 species are known and about 80 are considered poisonous or venomous (3, 4, 26). The starfish possess glandular tissue embedded in calcite, which secretes a toxin; the sea urchins possess a pedicellaria venom apparatus; and the sea cucumbers possess their toxin in specialized tubules which can be eviscerated.

Considering the starfish or asteroidea first, some nine species in six families are well known to be toxic (4). While most of the reports pertain to toxicity on ingestion, and dermatitis on handling, only *Acanthaster planci* is known to be venomous. Little is known of the symptomology in humans when poisonous starfish are ingested and most experiences deal with descriptions of local effects produced by handling these organisms.

Of the materials isolated and studied, most are saponin-type compounds which display the classic biological effects of this chemical group, *viz.*, hemolytic properties, and varying toxicities in low dosage. Examples include Asterosaponin A and B from *Asterias amurensis* and various other saponins from *Pycnopodia helianthoides*, *Asteria forbesi*, *Pisaster ochraceous*, *Pisaster brevispinus*, and *Patiria miniata* (232–242). Some of these saponin toxins show peculiar effects in that they are capable of causing sperm immobilization and induction of egg and sperm-shedding effects in related marine organisms (241). These substances certainly will be of value in studies related to sperm inactivation and egg maturation.

Another unique effect is noted in the capability of certain starfish isolates (*Asterias glacialis*, *Pisaster ochraceous*) to initiate an escape response in molluscs (242). *Pisaster ochraceous* also yields an invertebrate insulin (2, 243). A muscle-contracting principle from *Asterias rubens* has been also described by Fänge (239).

In the sea urchins (Echinoidea), the small globeriferous pincer-like organs (pedicellaria) are distributed over the entire body surface and serve as the venom organ (3). As would be expected, the toxic component apparently varies in the different sea urchins. Mendes (244), for example, has isolated a dialyzable, alkaline-sensitive, heat-labile toxin from the pedicellariae of *Lytechinus variegatus*, while Alender (237) has isolated a nondialyzable protein, which is pH-stable and heat-labile from pedicellariae of *Tripneustes gratilla*.

Sea urchins have shown toxicities on ingestion, and effects varying from simple diarrhea to death have been reported (4). The severe poisonings occur when the

ovaries are ingested, particularly during the reproductive period. Those species capable of producing poisonings include *Paracentrotus lividus*, *Tripneustes ventricosus*, and *Centrechinus antillarum*.

The action of the pedicellaria venoms and gonad extracts generally include symptoms of respiratory distress, muscle paralysis, convulsions, and death, both in vertebrates and invertebrates (4). These include certain molluscs, arthropods, mammals, and reptiles (3, 4, 245). Specific pharmacological effects noted are contraction of isolated guinea pig ileum by *Tripneustes gratilla* toxin (246); wide hemolytic activity against human, guinea pig, beef, rabbit, sheep, and fish erythrocytes; cardioactive properties; and blockage in response to indirectly stimulated muscle (3, 237, 247). The two latter properties indicate potential for the development of new cardioactive and neuromuscular agents.

In the class holothuroidea (sea cucumbers), there are about 1,100 species. At least 30 species are toxic. These are generally shaped like the common cucumber, have an average length from 2.54 cm. (1 in.) to 30.48 cm. (1 ft.), show from 10–30 small tentacles surrounding their mouths (to rake in sediment and food), and usually are found creeping slowly on the sea bottom or burrowing in the sand. They possess special defense organs, the Cuvierian tubules, which arise from a common stem of the respiratory tree (26). When provoked, the sea cucumber emits these tubules through its anus, causing ensnarement of the attacking animal in the long, extremely sticky thread-like tubules. During this process a potent toxin is also discharged.

It is interesting to note that the toxicity of these organisms has been well-known for a long time by natives in the South Pacific islands. They use the juice squeezed from certain species to poison pools along coral reefs to catch fish for food. Several species are highly valued as food, being sold on the Oriental market as Trepang. Repeated boiling or digestive enzymes are apparently effective in destroying the toxins (20).

Study of the toxins isolated from the viscera and Cuvierian tubules of *Actinopyga agassizi*, show these toxins to be a mixture of steroidal glycosides, the first steroid saponins of animal origin (2, 248). The toxins have been characterized as a mixture of at least six water-soluble glycosides. On hydrolysis, the four monosaccharides, glucose, xylose, quinovose, and 3-*O*-methylglucose, are yielded from Position 3, Ring A of the steroid nucleus, in addition to a mixture of steroid aglycones (holothurinogenins). Molecular weight determinations give values for C_{30}-steroid tetraglycoside sulfates. One of the fractions in the mixture, called holothurin A, and its genin have been characterized (2, 249–254).

Another fraction, designated as holothurin B, has been obtained from the steroidal glycoside mixture present in *Holothuria leucospilota* by Matsuno *et al.* (255, 256). It has been characterized as the sodium salt of a sulfated triterpenoid aglycone having quinovose and xylose as sugar moieties. Holothurin B has the empirical formula, $C_{41}H_{65}O_{13}—OSO_3Na \cdot 2H_2O$ and melts with decomposition at 223–224°.

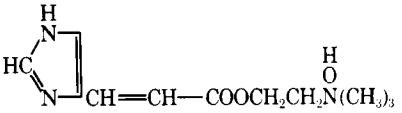
The holothurins show a wide variety of pharmacological actions. They have been found to be toxic to a wide variety of animals which include crustaceans, sea anemones, earthworms, molluscs, fish, and mammals (4, 253, 257–259); in addition, they show hemolytic properties (258, 260); cytotoxic and neuromuscular effects (261, 262); neurotoxic effects (263, 264); antitumor properties (2, 24, 258, 259, 265, 266); and abnormal effects on the normal development of sea urchin eggs (267). Crude holothurin has a lethal dose (IP) of 0.2 mg./mouse (265). Since many steroidal glycoside drugs like those from *Digitalis* species and other plants have proven value as drugs, perhaps the holothurins will someday be useful as possible neuroactive or antitumor drugs.

A recent review of the pharmacology of substances elaborated by the starfish, sea urchins, and sea cucumbers is given by Alender and Russell (268). Here one may also find an excellent summary of the various neurosecretions and neurohumors and other active substances (3). Briefly, homarine (269–271), serotonin (272), the phosphogens, phosphoarginine and phosphocreatine (273), steroids (274), mucopolysaccharides (275), acetylcholine-like compounds (276, 277), saponin-like substance (235), holothurins (248–254), arylsulfatase and β-glucuronidase (278), and amine oxidases (279, 280) have been either isolated or described in many members of the echinoderms. Many of these have biomedical import.

Mollusca—The molluscs represent a large group of marine organisms having about 80,000 species. Eighty-five or so species have been known to poison man either upon ingestion or by way of a venom apparatus. This phylum includes such diverse animals as oysters, snails, clams, abalones, mussels, and octopuses. Their characteristics include a calcareous shell with an underlining mantle of tissue, a gut with two openings, a body cavity, and a ventral muscular foot. They range in size from under an inch (small shellfish) to over 50 ft. (giant squid).

Most of the poisonous and venomous species are found in three of the five classes of molluscs. These include the gastropods (univalves), pelecypoda (bivalves), and cephalopods (octopuses, squid) (3, 4). While many of the shellfish can produce poisoning on ingestion, this is usually due to prior ingestion of certain members of the Protista, *e.g.*, toxic dinoflagellates, algae, *etc.* This aspect of shellfish poisoning has already been discussed under the original causitive microorganisms.

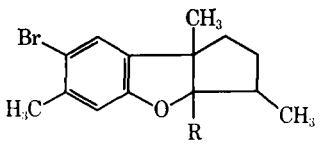
Of the nonvenomous gastropods which contain toxins producing deleterious effects on ingestion and other effects, several species are of particular interest. The toxin tetramine is present in the salivary glands and saliva of *Neptunea arthritica* (281); the toxin murexine {urocanylcholine or β-[imidazolyl-(4)]-acrylylcholine} (IX), is found in the hypobranchial gland of *Murex*



IX

species (282); the active principles senecioylcholine and acrylylcholine have been identified in *Thais floridana* and

Buccinum undatum (283); a photodynamic pigment toxin related to chlorophyll has been reported in the abalone *Haliotus discus* (284); a cholinergic toxin is described and two heterocyclic aromatic bromo compounds, aplysin and aplysinol (X) have been isolated



X
(Aplysin, R = CH_3)
(Aplysinol, R = CH_2OH)

from the sea hare, *Aplysia kurodai* (285, 286); extracts of molluscs have been described which regulate embryonic motor activity (287); and a neurohormone and cardioaccelerator are described in the snail, *Helix aspersa* (288, 289).

Tetramine has a curare-like effect on frogs and in mammals in addition to producing hypotensive states and bradycardia. Probable phrenic-nerve paralysis causes temporary respiratory paralysis when tetramine is injected intravenously (3, 281, 290).

Murexine effects have been long known. It has toxic and paralyzing effects on fish, amphibia, and some invertebrates. Ganglion-stimulating and neuromuscular-blocking action (*via* depolarization), attributed to this substance, help explain these effects (2, 17).

Senecioyl choline (β,β-dimethylacrylyl choline) has pharmacological action similar to murexine. Its effects include excitation of the carotid sinus receptors, producing respiratory stimulation and excitation of sympathetic ganglia. In addition, it can produce neuromuscular block. The other choline derivative, acrylyl choline, possesses neuromuscular-blocking properties and shows nicotinic action (2, 17).

Aplysin has shown hypotensive properties in dogs and can produce muscle contracture and a cardiac-stopping effect in frogs. Oral feeding in mice causes rapid hypersalivation, hyperventilation, ataxia, loss of motor coordination, respiratory paralysis, and ultimately death (285, 286, 291).

The uncharacterized photodynamic pigment from abalone appears to be present in the livers and viscera of the organism. After ingestion of these, and subsequent exposure to light, such dermatological effects as burning, stinging, itching, edema, and skin ulceration have been observed in man. Experimental work on cats, rats, and mice, which were fed the toxin and exposed to sunlight, showed salivation, lacrimation, and in some with high sensitivity, convulsions, followed by paralysis and death in 30 min. (284).

The choline derivatives mentioned above seem to point the way to new neurotropic and CNS drugs while aplysin derivatives may have use as hypotensives or cardioactive agents. Continued research on the photodynamic pigment in abalone may lead to a better understanding of the mechanism of drug-induced photosensitivity.

An interesting series of reports by Li *et al.* (2, 292–298) have indicated that a wide variety of molluscs possess antiviral and antibacterial substances. These were generated by the observation that the fluid in commercially available canned abalone products had antimicrobial activity. The antimicrobial fraction has been designated as paolin I (paolins refer to the Chinese name for abalone) and the antiviral fraction has been designated as paolin II. They are separable by cellulose ion-exchange chromatography. Both are relatively thermostable (95° for 45 min.) and, because they are nondialyzable, appear to be protein in nature. Pepsin, however, does not digest them. Paolin I appears to be a mucoprotein with a molecular weight of 5000–10,000. Another fraction, designated water-soluble Fraction C, is described and can be obtained from both abalones and oysters. It is prepared by homogenization, acidification, dialysis, and lyophilization of these and it possesses both antibiotic and antiviral activity.

It is significant to note that all of these preparations possess potent antibacterial and antiviral activity *in vitro* as well as *in vivo*. Both paolin I and Fraction C were found capable of reducing the death rate (by 27%), of mice experimentally infected with *Streptococcus pyogenes*. In addition, growth inhibition both *in vitro* and *in vivo*, has been noted with a penicillin-resistant strain of *Staphylococcus aureus*.

When paolin II and Fraction C were used to treat monkey kidney tissue 24 hr. prior to infection, a 99.9% inhibition of poliovirus and influenza A virus growth was noted. Also, Fraction C protected mice *in vivo* against experimentally induced infections of poliovirus and influenza B virus.

Tissue culture experiments showed similar growth inhibition against *Herpes simplex*, keratitis virus, adenovirus type 12, and tobacco mosaic virus. No apparent toxicities of the crude extracts or paolins have been experienced.

The organisms thus far shown to contain these principles include the abalone (*Haliotis rufescens*), the oyster (*Crassostrea* spp.), the squid (*Loligo pealii*), the queen conch (*Strombus gigas*), the common clam (*Mercenaria mercenaria*), and the sea snail (*Tegula gallina*).

In addition to antimicrobial and antiviral activity, certain molluscs also show antitumor properties. It was recently noted that extracts of the common edible clam, *Mercenaria mercenaria*, can inhibit growth of tumors. The active principle has been designated as mercenene and it is described as a water-soluble, heat-stable, poorly dialyzable glycopeptide with a molecular weight of 1,000–2,000 (299–302). The activity apparently varies with temperature and summer clam tissue extracts show an activity eight to nine times winter clam tissue extracts (297).

The antitumor activity of mercenene has been shown against Krebs-2-carcinoma, Krebs-2-ascites, and sarcoma 180 even when administered several days after tumor implantation. Carcinolytic activity against a human HeLa cell line *in vitro* has been noted also. Mercenene had no effect on the growth of a normal human amnion cell line, and it is apparently nontoxic at the therapeutic levels in mice.

Turning now to the venom-type of intoxication which occurs on being bitten or stung, certain members of the

classes Gastropoda and Cephalopoda of the Mollusca will be considered. The ability of several genera of octopuses to paralyze prey by means of secretions of the posterior salivary glands is well known. These have been reviewed by Nigrelli (2), Russell (3), and Halstead (4).

Many species of octopus (*e.g., Octopus vulgaris, O. macropus*) feed largely on crabs or shellfish which are captured with their tentacles. Although one might assume that they bite their prey with their powerful beaks or pull the shellfish apart with their tentacles, this is not the case. They inject the secretion of their posterior salivary glands over the organism or through a rasped hole in the shell and thus paralyze and relax it. In the case of the shellfish, this enables the octopus to remove it easily from its shell for ingestion.

The studies on the substances contained in the salivary glands reveal an impressive array of active compounds including guanidine bases, 11-hydroxysteroids, polyphenolic compounds, an enormous number of different amines, and certain pharmacologically active peptides and proteins.

Some of these amines include tyramine, octopamine (1-*p*-hydroxyphenylethanolamine), octopine, agmatine, acetylcholine, adrenaline, noradrenaline, phenolamines, indolamines, 5-hydroxytryptamine, histamine, dopamine, tryptophan, 3,4-dihydroxyphenylserine, 3,3-dihydroxyphenylalanine, 3,4-dihydroxyphenylethylamine, and *m*-hydroxyphenylalanine (303, 304). At least 15 different enzymatic activities are noted in the saliva of *Octopus vulgaris* (3), these serving a digestive function.

Even though many of these have strong physiological activities, none produce paralytic effects. The paralytic effects have been attributed to a protein named cephalotoxin. This is thermolabile, inactivated by trypsin, and is felt to be a glycoprotein (304). So even though the crude venom of octopus salivary gland induces paralysis in several animals (fish, crabs, shellfish, frogs, rabbits), the stimulant effects are probably induced by the bioactive amines and peptides while the paralytic effects are produced by a toxic protein such as cephalotoxin.

Studies on cephalotoxin itself indicate potent effects throughout the various physiological systems. It causes a decrease in amplitude and produces arrest in diastole of isolated perfused octopus and crab hearts; it increases the amplitude of contraction of the duodenum and inhibits respiration in rabbits; and it can produce complete paralysis in crustacea at a concentration of 0.1 mg./g. in less than 60 min. (304, 305).

Another interesting active substance obtained from the posterior salivary glands of *Eledone moschata* and *E. aldrovandi* is eledoisin. This is a methanol-soluble polypeptide, specifically an endecapeptide, having the following linear amino acid sequence: pry-pro-ser-lys-asp(OH)-ala-phe-ileu-gly-leu-met-NH_2. When injected into mammals, it causes marked vasodilatation, hypotension, and stimulation of certain extravascular smooth muscles. At a dosage level of 300 mcg./kg. (s.c.) eledoisin is lethal to mammals (3, 306).

Later experiments showed that eledoisin is a potent hypotensive in dogs, possessing at least 50 times the potency of histamine, acetylcholine, and bradykinin in provoking this response. In addition, it increases permeability of peripheral vessels, stimulates gastrointestinal tract smooth muscle, and produces an atropine-resistant increase in salivary secretions (3, 307, 308).

Cephalotoxin and eledoisin could lead to useful CNS and hypotensive drugs, respectively, particularly if research continues on their mode of action.

In the Gastropoda, the last class to be considered here in the molluscs, the most venomous are species of the genus *Conus*. At least 15 species are known to be toxic to man (4, 26). These organisms possess a variously colored cone-shaped shell and are mostly shallow-water inhabitants in tropical and subtropical seas. They possess a unique and remarkable venom apparatus. A long tubular venom duct, terminating in a muscular venom bulb, arises from the pharyngeal region of the food canal. The cells lining the lumen of the venom duct form microscopic sausage-shaped bodies containing toxic material. A protrusible and mobile proboscis is formed by extension of the anterior end of the pharyngeal region. Miniature hollow-barbed harpoons, formed and stored in a radular sac off of the pharynx, are transferred to the tip of the proboscis and held there. Prior to being thrust into the prey, a contraction of muscles in the walls of the venom bulb forces fluid containing venom bodies along the duct and proboscis and through the harpoon. The harpoon thus acts like a hypodermic needle or dart which is injected into the prey with a dose of the venom. Its venom relaxes the musclature of the prey causing it to hang loosely from its shell. The prey is then enveloped by the distensible proboscis and easily ingested. Most species of *Conus* prey on molluscs, worms, and certain fish (28).

The venom obtained from *Conus* is viscous, has a pH range of 7.8 to 8.1, and varies in color from white to yellow to gray or black depending on the species. It has been extensively studied by Endean *et al.* (309–316). As with other venoms it is a mixture of active amines, peptides, and proteins. Compounds identified include *N*-methylpyridinium, homarine, γ-butyrobetaine, 5-hydroxytryptamine, lipoproteins, carbohydrates, several amines, and several possible indole amines. The active toxin is nondialyzable, only slightly thermolabile, and unaffected by treatment with trypsin. Endean *et al.* (312) report that the venom bodies or granules possess a peripheral film of polysaccharide, a sheath in which protein and lipid are found and a core in which protein and 3-indoyl derivatives are found. Even though the exact nature of the lethal component of *Conus* venom is unknown, a fivefold purification of the toxin by gel-filtration techniques using Sephadex G-200 indicates that it is a protein (314).

Injection of some *Conus* venoms experimentally in various animals produces increased parasympathetic activity in addition to muscular weakness, alterations in the deep reflexes, ataxia, and tremors. Larger doses produce paralysis of skeletal muscles, convulsions, respiratory arrest, and symptoms of cardiac failure.

Endean (312) reports that the venom of *Conus striatus* has a direct effect on diaphragm musculature which causes a reversible progressive decline in twitches. It was noted that this effect was occasionally accompanied by a sustained contracture of the muscle. Neither neostigmine nor eserine had any effect on the paralysis.

The lethal dosage range for the venom of *Conus*

varies from 0.2–1.3 mg./kg. body weight for *Conus geographus* and *Conus magnus* to over 200 mg./kg. for *Conus stercusmuscarius* (3, 310, 316). Human fatalities have also been reported after envenomation by *Conus* species (4, 28).

If sufficient studies show that the active principle of venom of *Conus* has no adverse effects on cellular processes it is possible to assume that it might yield a valuable muscle relaxant. If nothing else, these principles serve as tools in advancing our knowledge of the mechanism of muscular contraction.

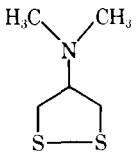
Lastly, one cannot overlook pharmaceutical products using calcium carbonate derived from oyster shells. At least one pharmaceutical company has over eight products containing this "Os-Cal" line (Marion Labs., Kansas City, Mo.).

Annelida (Segmented Worms)—In this phylum the class Polychaeta has about 4,000 species which are common but rather inconspicuous marine creatures. The polychaetes usually burrow in sand or mud and are frequently found under rocks. They have elongated, usually segmented bodies with paired setae. About nine species are known to be toxic.

According to Halstead (4) very little is known regarding the toxicity of these organisms. Some have pungent parapodial bristle-like setae and at least one species, *Gycera dibranchiata*, is capable of inflicting a venomous bite.

The major annelid of biomedical interest is the Japanese species, *Lumbriconereis heteropoda*, from which the potent neurotoxic agent, nereistoxin is obtained. Investigations here were stimulated by the observation that carnivorous insects died from feeding on the dead bodies of this annelid.

Nitta (317) in 1934, first isolated and partially characterized this principle, and Hashimoto *et al.* (318, 325, 326) later completed the elucidation of its structure and confirmed its powerful insecticidal activity. The structure of nereistoxin was confirmed by synthesis (324) and it is *N,N*-dimethylamino-1,2-dithiolane (XI) (326).



XI

Although the mechanism of action of nereistoxin has not been completely clarified, Sakai (323) found that it paralyzed the adult male American cockroach, *Periplaneta americana*, by effecting a ganglionic-blocking action on its central nervous system. It has also been found to be an effective insecticide against houseflies, rice stem borers, soybean aphides, and many other agricultural pests.

Because of the chemical instability of nereistoxin, more than 20 related compounds were synthesized before a stable modification could be obtained. This led to the development of 1,3-bis-(carbamoylthio)-2-*N,N*-(dimethylamino) propane which is marketed in Japan under the trade name Padan by the Takeda Pharmaceutical and Chemical Co. It is widely used against the rice stem borer and serves as yet another example of how a natural product can lead to a useful new synthetic bioactive molecule.

Further study on the nature of the neurotoxicity and cardiotoxicity of nereistoxin against insects, mice, fish, rabbits, and monkeys may lead to a useful neuroactive drug for humans.

Sipunculida (Peanut Worms)—These are a group of unsegmented, wormlike invertebrates. The body is differentiated into an anterior slender introvert and a posterior plump cylindrical trunk with tentacular outgrowths encircling the terminal mount. The biology, toxicology, and history of research on these organisms, which include species of *Bonellia* and *Golfingia*, has been reviewed by Halstead (4).

Bonellia viridis and *Bonellia fulginosa* have been found to contain toxic substances. One toxin, which is water- and ethanol-soluble and dialyzable, is called "bonellin" and has been obtained from the proboscis of the former organism. Crude extracts of *Bonellia* have deleterious paralytic effects on protozoa, nematodes, annelids, crustacea, and tadpoles. Bonellin has shown sperm immobilization and development-arresting effects on the sea urchin, *Arbacia punctulata* (327). The masculinizing effect of bonellin has already been mentioned (2, 20) earlier in this paper. Although bonellin is apparently nontoxic to fish and rabbits, its paralytic and growth-retardant effects on lower forms indicate potential for neuroactive and/or development-affecting agents.

Platyhelminthes (Flatworms)—These are a group of worms which are characterized by a flattened body, gut with a single opening, and no body cavity. Of the three classes in this phylum, only the class Turbellaria is of marine or fresh-water occurrence, although some species are found on land. For the most part, members in this class are free-living and possess cilia which lash and aid in propulsion. About 1,600 species are described in the class.

Halstead (4) has reviewed the toxicity of some of the members in this group. They include the fresh-water turbillarians, *Dendrocoelum lacleum*, *Polycelis nigra*, *P. cornuta*, *Planaria gonocephela*, *P. lugubris*, and *Bdellocephela punctata* which are toxic to a variety of laboratory animals (328).

Some marine species include *Leptoplana tremellaris*, *Stylochus neapolitanus*, *Thysanozoon brocchi*, *Yungia aurantiaca*, *Procerodes lobata*, and *Bdelloura candida*. Saline extracts of these, on intracardial injection into guinea pigs, caused death in 1 to 40 min. The toxicity of the extract could be destroyed by heating it at 100° for 1 min. (329). Further, extracts of the first five species in this paragraph effected cardiac arrest (in systole) within 1 to 25 min. when added to isotonic frog Ringer's solution containing a prefused isolated frog heart. Perfusion with fresh Ringer's solution could reverse the effect. Smaller amounts of the extracts caused an increase in the amplitude of the contraction which either returned to normal or produced cardiac arrest in systole or diastole. Solution of atropine failed to reverse these effects (329).

Apparently little has been done on turbellarian poisons since these early reports which indicate potential for cardioactive agents.

Nemertinea (Ribbon Worms)—This phylum, also referred to as Rhynchocoela, includes many of the familiar unsegmented worms found along the seashore. They are characterized as being ribbon-shaped, having a gut with two openings, no body cavity, and a venomous barb-tipped proboscis which turns inside out when projected. Of the estimated 550-odd species, most are marine with few fresh-water and land species.

Halstead (4) has reviewed the toxicology of this phylum. Crude alcoholic extracts of *Amphiporus lactifloreus* and *Drepanophorus crassus*, injected into the lymph sacs of frogs caused increased respiration, dilated pupils, and extension of limbs, followed by recovery in 30 min. When injected into crabs, excitation, prostration, and death occurred (330).

A substance, amphiporine, which is considered to be an alkaloid has been isolated from the aforementioned species and the effects described above are attributed to it. In addition, it appears to have a paralyzing effect on sympathetic ganglia.

Another substance, nemertine, has been described and isolated from *Lineus lacteus* and *L. longissimus* and found to be a nerve stimulant. It is less toxic than amphiporine.

Although the substances, amphiporine and nemertine, appear to be devoid of remarkable pharmacological activity, continued work on their characterization as well as the venoms of this group should lead to useful neuroactive drugs.

Arthropoda (Joint-Footed Animals)

This phylum makes up around 80% of all known animals possessing some 775,000 species. Those of interest here include members of the class Merostomata (houseshoe crabs) and the class Crustacea (crustaceans). The phylum is characterized by individuals which have a chitinous skeleton, jointed legs, and a segmented body. Relatively few, *viz.*, some eight species have been reported to contain toxic materials.

Halstead (4) has reviewed the medical aspects of intoxication in this group. Generally, toxicity is related to ingestion at certain times of the year particularly during reproductive cycles of the animals. Examples include species of the Oriental horseshoe crabs, *Carcinoscorpius* and *Tachypleus*. Characteristic gastrointestinal and severe neurological symptoms are noted. Little is known regarding the substances responsible for these effects.

Of greater biomedical interest in this group are the extracts of the crustacean nerves and hearts. These have nerve-blocking properties, *e.g.*, the agents found in the lobster, *Homarus americanus* which include γ-aminobutyric acid (GABA), betaine, and taurine (331); cardiac-depressing properties, *e.g.*, homarine (332); cardioaccelerator substances, *e.g.*, 6-HT and a mucopeptide from *Carcinus maenas* (333); and inhibitory, hatching, and other substances from other species (334–337). These certainly represent potential leads to neuromuscular and cardiac-affecting drugs.

Of recent popular interest is the fact that certain species of barnacles (crustacea) are being examined as possible sources of cements or adhesives for glueing fillings permanently in human teeth. It is well-known that when a barnacle is removed forcibly from steel, small particles of the metal often are removed with it, indicating that the break occurred in the metal. Current experiments indicate that barnacles bond just as forcibly to dead teeth which were experimentally mounted in plastic and left in tropical waters containing the organisms.

Extraction of the uncured cement from the barnacles followed by its characterization should yield new and possible synthetic routes to biomedically important marine-derived adhesives (338).

Vertebrates

Fish—According to Russell (3, 26) approximately 500 species of marine fish are known to be toxic. It is, therefore, impossible to review all of these, but for the sake of convenience, certain important representative species are given in Table I.

Generally, fish may be considered to be in two major groups, *viz*,, those which contain a poison within their musculature, viscera, skin (ichthyosarcotoxic), gonads (ichthyootoxic), or blood (ichthyohemotoxic) and those which are venomous.

Fish are placed in three classes, the Agnatha or jawless fish, the Chondrichthyes or cartilaginous fish, and the Osteichthyes or bony fish. For the purposes of this review, the last class is divided into certain groups using the classification system of Russell (26).

Prominent in the Agnatha is the hagfish, *Eptatretus stoutii*, which yields a low molecular weight aromatic amine named eptatretin. It was isolated from the aneural branchial heart of this organism and has shown potent cardiac stimulant and pacemaker activity on the frog, dog, and on *Eptatretus* heart itself. Injected into dogs with heart failure due to myocardial ischemia, it greatly improved ventricular work capacity (339, 340).

Certain cartilaginous fish, including the sharks and stingrays, show wide pharmacological activity due either to ingested livers and musculature (sharks) or venom (stingrays). The shark toxins may be a form of ciguatera poisoning while the proteinaceous venom of the stingray (*Urobatis halleri*) produces slight CNS and marked cardiac effects (26).

Work by Russell (3), on *Urobatis halleri* has shown the toxin to be a protein with a molecular weight over 100,000. In addition, the venom contains serotonin, 5-nucleotidase, and phosphodiesterase. It is capable of producing changes in heart rate and amplitude of systole and may often produce complete, irreversible cardiac standstill. Separation of the protein into several fractions has been undertaken and perhaps one or more components may eventually yield new cardioactive molecules with highly specific activities. Several references on other stingrays and reviews on related animals in this group are cited by Russell (3, 341).

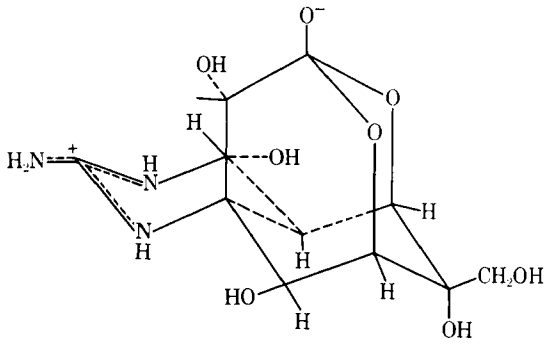
In the Osteichthyes, one of the largest group of ichthyosarcotoxic fish are those implicated in ciguatera poisoning. This is a type of toxicity characterized by gastrointestinal and neurological effects following ingestion. There are over 30 species in 12 families including, for example, the sturgeon fish, sea basses, snappers, barracudas, *etc.* There is a wide range of pharmacological effects seen in toxicities here and the toxin is

felt to originate in certain toxic algae. Herbivorous species feed on toxic algae, carnivorous species feed on the herbivores, and somehow the toxin is accumulated, concentrated, and passed on from fish to fish.

Scheuer (342) considers ciguatoxin to be an unstable lipid compound containing carbonyl and hydroxyl functions and a quaternary nitrogen. The empirical formula is given as $C_{35}H_{65}NO_8$. Other investigations on ciguatera toxin include work by Hessel (343), Banner (344, 345), Li (346), and McFarren (347).

Other toxic components considered as ichthyohemotoxins and ichthyosarcotoxins, include a proteinaceous substance in the serum of the eel, *Anguilla vulgaris* (348) and toxic lipoproteins and lipids in the roe of the blenny *Stichaeus grigorjewi* (349, 350). Further characterization of ciguatoxin and other related toxins may lead to neuroactive and gastrointestinal-active agents.

Perhaps the most spectacular single substance of pharmacological interest isolated and characterized in the past few years from marine sources is tetrodotoxin (XII), the puffer or fugu poison. It is found uniformly in



XII

the Diodontidae (porcupine fish, 10 spp.), the Molidae (sunfish, 1 spp.) and Tetraodontidae (puffers, 40 spp.), and recently in the California newt *Taricha torosa* (amphibian) and is the most toxic of the ichthyosarcotoxic poisons. Chemical studies show that it is an aminoperhydroquinazoline compound with a molecular formula of $C_{11}H_{17}N_3O_8$. The chemistry and pharmacology have recently been reviewed by Kao (351), Russell (3), and Nigrelli (2).

Basically, tetrodotoxin blocks conduction in nerves exerting its most deleterious effect on the motor axons and on the muscle membrane. In addition it blocks the excitability of directly stimulated skeletal muscle fibers. The way it does this is unique because apparently the selective effect of it is on the axon membrane and *not* on the ions which carry current inward. In other words, the membrane fails to become highly permeable to sodium ions and their passage is blocked. This blocks conduction in nerves.

Further evidence is provided that the sites of block of neuromuscular transmission are the nerve axon and muscle membranes and *not* the endplate receptors. This means that conduction in nerves and muscle membranes is basically different from that in the synapse and that the electrically excitable part could be blocked without greatly affecting the chemosensitive part (3, 351, 352).

When the structure activity relationships are worked out with tetrodotoxin, it should be possible to synthesize new highly selective local anesthetics and other neuroactive drugs.

In small doses, tetrodotoxin has been used clinically to relax muscular spasms and as a palliative in terminal cancer (21). It is available from Sankyo Co., Tokyo, Japan, and Calbiochem Co., Calif., in the United States.

The scombroid and clupeoid groups of fish shown in Table I, do not seem to hold much promise as potential sources of drugs, but perhaps this is because little is known of their toxic principles. Some CNS, gastrointestinal, and local activity is noted in the poisons of the schombroid group, *viz.*, mackerel-like fish, tunas, skipjacks, and bonitos (26).

Such is the case also with the mullet and surmullet (goatfish) of the group of fish which are reported to cause lightheadedness, hallucinations, depression, and violent nightmares. Certainly, further studies should yield new CNS-active drugs particularly psychotomimetics (26).

In the last important group of fish, those possessing poison glands with venom show much promise of yielding active materials. According to Russell (3), over 220 species of fish which include the stingrays (mentioned above), zebrafish, scorpion fish, weevers, stonefish, stargazers, and certain members of the sharks, catfish, ratfish, and sturgeon fish, are described or felt to be venomous.

For the most part, the venomous fish are found in shallow-water reef areas or close to shore. The stingrays and stargazers are mostly benthonic but may occasionally be found in deep waters. Most are also nonmigratory, slow swimming, and use their venom apparatus defensively. Halstead (4) has reviewed the various venom organs in Volumes two and three of his books.

The venoms differ quite markedly from the poisons described earlier and are generally quite labile or instable and are usually complex mixtures of various molecular weight peptides, proteins, various enzymes, and other principles. Apparently only certain fractions in a given protein mixture are the true toxic components. Russell (3) notes that no basic structure for the toxin of any venomous fish has yet been determined. However, there is enough apparent similarity in the pharmacological properties of the venoms of weevers, scorpion fish, and stingrays to suggest that they may contain similar toxic constituents.

Some of the interesting venoms include the proteinaceous toxins of the weeverfish (*Trachinus draco* and *T. vipera*) which decrease blood pressure, respiration, and heart rate (26, 353–355); the proteinaceous toxins of the scorpion fish (*Pterois volitans*, *Scorpaena guttata*) which produce muscular weakness, hypotension, paralysis, depressed respiration, and death (26, 356); and the proteinaceous toxin of the stonefish (*Synanceja horrida*) which has direct paralyzing effects on cardiac, skeletal, and smooth muscle (357–360).

With respect to the relative potencies of these venoms, weeverfish venom has an LD_{100} in mice of 0.0004 ml./17-g. mouse (361), zebrafish (*Pterois volitans*) has an LD_{50} in mice of 1.1 mg. protein/kg. mouse (356), and stonefish has an LD_{50} in mice (i.v.) of 0.4–0.6 mcg. protein/kg. mouse (26, 359).

Although relatively little has been done on the full

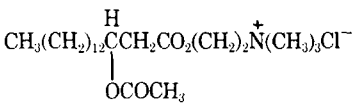
characterization of the numerous components of these venoms, it appears that a wealth of highly specific bioactive macromolecular peptides and/or proteins may be yielded on further research. Thus far, interesting cardiovascular and muscle-relaxing effects of some of the venoms give some hint of potentially useful drugs.

Besides the ichthyosarcotoxic and venomous fish discussed above, there are others which secrete toxic substances (foams or mucus) into their immediate surroundings when disturbed or attacked. One of these is a peptide-type toxin secreted by the Pacific grouper (*Grammistes sexlineatus*). It is toxic to other fish and sea urchins. Low concentrations (50–100 p.p.m. of toxin) immobilize sea urchin sperm, affect embryological development of sea urchin eggs, and cause cytolysis of their unfertilized eggs. In addition, it has antibiotic properties in that it is capable of inhibiting the growth of *Escherichia coli* (362).

Another interesting fish in the same family, known as the soapfish (*Rypticus saponaceous*), also secretes a peptide-containing toxic mucus. This has been shown to be toxic to mice, causing motor disabilities, and toxic to guppies, causing loss of sense of equilibrium prior to death (363).

Although these examples of the Grammistidae family show active materials, more work on toxic mucous secretions seem to have been carried out on the members of the Ostraciontidae. This is the well-known trunkfish family whose members are called boxfish. It has been long known that the Hawaiian boxfish, *Ostracion lentiginosus*, secretes a substance which rapidly kills fish in its vicinity.

Early work by Thomson (364) showed that a crude preparation from these fish, which he called ostracitoxin, was indeed toxic. Based on similarities in detergent and hemolytic activities he thought that the toxin was related to the steroidal saponins isolated from echinoderms. However, subsequent studies by Boylan and Scheuer (365) showed that it was not a steroidal saponin. They isolated and characterized the toxin, which was renamed pahutoxin, as the choline chloride ester of 3-acetoxyhexadecanoic acid (XIII). The isolated and synthetically prepared product was shown to be comparable in lethality and hemolytic properties to the natural material.



XIII

Although preliminary synthesis of a few related compounds has not yielded any useful pharmacologically active substances, the unique structure and activity of pahutoxin opens the way for a host of potentially useful synthetic derivatives based on a new type of structure. This investigation represents the first chemical identification of a toxic secretion by a marine organism. The structural elucidation of the active principles of the previously mentioned toxic secretions may lead to neuroactive drugs.

With respect to other general effects of these substances secreted into the marine environment, it has been noted that they may be growth-stimulating as well as growth-inhibiting (12, 366). It has been found that extracts of goldfish mucus (*Carassius auratus*) at dilutions of 1:400,000–1:800,000 are effective growth stimulants for developing goldfish and even developing arthropods (367).

Other agents elaborated into the aquatic environment by certain organisms particularly in the face of danger are interesting in that they act as true alarm substances. They are capable of evoking fright reactions, like confusion, agitation, and hiding responses. Apparently certain species of fish and other marine organisms possess olfactory or other sensing mechanisms for detecting these elaborated materials at great dilutions (368).

Many interesting and new hormones have been isolated from various parts of fish. From the neurohypophysis, vasotocin (8-arginine oxytocin) has been universally found (369, 370), while isotocin or ichthyotocin (4-serine, 8-isoleucine oxytocin) seems to be limited to only bony fish and some primitive forms including the African bichir and lungfish (371, 372). These latter two species have also yielded a new hormone similar to 8-isoleucine oxytocin. It appears to be present also in amphibians but absent from other bony fish and has shown oxytocin properties on isolated rat uteri (371, 372). Another unique hormone, glumitocin (4-serine-8-glutamine oxytocin), has been isolated from the neurohypophysis of the cartilaginous ray fish, *Raja clavata* (373). The characterization of the neurohypophyseal hormones of the fresh-water carp are also described and compared with the hormones of bony marine fish (374).

The neurointermediate lobes of the pituitaries of certain elasmobranch species have yielded a new hormone which has oxytocic properties (375). Another pituitary extract from the carp has been described which contains a unique glycoprotein-type gonadotrophic factor. It has several effects including influences on the spermiation in the frog and on the metabolism of eel testes. It has no activity on mammalian preparations (376).

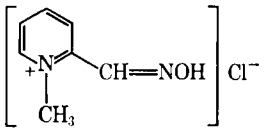
The urohypophysis (caudal neurosecretory organ) of *Tilapia mossambica* has yielded at least two hormones with antidiuretic activity (377). The same organ of *Cyprinus carpio* and other species have yielded a cholinergic substance (378).

Special species of fish contain in their gonads, many of the common and, in some cases, unique steroids. These include progesterone, estradiol-17β, estrone, estriol, androstenedione, and testosterone (379). Still others are found in the plasma of salmon, channel catfish, and various other fish species, *viz.*, testosterone 11-ketotestosterone, 16-ketoestradiol, estriol, estradiol-17β, estradiol-17α, epiestriol, estrone, 17α-hydroxyprogesterone, 20β-dihydro-17α-hydroxyprogesterone, cortisol, and cortisone (379–382).

In the pronephric and pharyngeal thyroid of the goldfish (*Carassius auratus*) thyroxin biosynthesis has been described by Chavin (383). The potential of these marine organisms as sources for the known hormones and new and different bioactive steroids is obvious.

For many years, the South American electric eel fish (*Electrophorus electricus*) has been the subject of intense

research particularly on how it produces and discharges over 600 v. of electricity when disturbed. Less well known is the fact that its organs are an extremely rich source of the enzyme cholinesterase. Research on the electric eel led to many discoveries and laid the groundwork for the synthesis of PAM (pyridine aldoxide methiodide) which is a potent antidote for pesticide poisoning. The fact that cholinesterase can be poisoned, that is, irreversibly bound to organophosphates so that it will be biochemically inactivated, is well known. When this happens, acetylcholine accumulates, producing increased salivation, lacrimation, bronchopulmonary secretions, sweating, vomiting, nausea, miosis, bradycardia, weakness, and fasciculations ending in apathy, paralysis, drowsiness, confusion, and finally, convulsions with coma and death. It was found that the most useful of the oximes capable of reacting with the inactivated cholinesterase to remove the inhibiting phosphoryl group and release cholinesterase in active form is 2-formyl-1,1-methyl-pyridinium chloride oxime or pralidoxime chloride (XIV) (30, 35). It is an effective cholinesterase reactivator.[3]



XIV

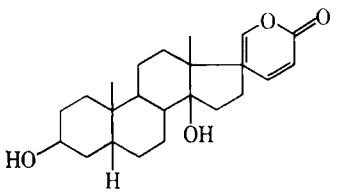
Amphibians—The amphibians form a separate class of vertebrates of their own. They are considered to fall somewhere between the fish, from which they arose, and the reptiles, to which they gave rise or evolved. For the most part, they depend on water in which to live and breed and to prevent dehydration. Amphibians are fishlike to the extent that their larval stages breathe through gills. When mature adults, their skin is characterized as moist and scaleless.

Relatively few in this group have yielded biomedically important substances, *viz.*, a few species of salamanders and frogs. However, the few that have indicate a necessity for closer examination of all members in the class.

Of particular interest is the fact that an early independent study by Twitty (352) showed that the grafted eye and limb buds from embryos of the California newt (*Taricha torosa*) onto the larvae of the striped salamander (*Ambystoma tigrinum*) caused paralysis of the host species. Subsequent studies showed that the eggs and embryos of *Taricha torosa* and other members of the Salamandridae family (*Notophthalmus*, *Cynops*, and *Triturus* spp.) contained a potent toxin called tarichatoxin. It was later shown that this was identical with tetrodotoxin (124, 352, 384–387). Since this interesting compound has already been discussed, further elaboration here is unnecessary. Why this unusual compound is found in one suborder of fish and one family of amphibians is unknown. It can perhaps be explained as an example of convergent evolution of some highly specific biogenetic pathway.

Another interesting series of pharmacologically active substances has been isolated from certain species of frogs or toads. It has been known for centuries that toads produce a poisonous secretion. Specifically it is elaborated and contained mainly in a pair of well-defined skin glands behind the eyes known as "parotid" glands (*not* to be related to the salivary glands).

The species here are all of the genus *Bufo* of the family Bufonidae (*e.g.*, *B. bufo bufo*, *B. marinus*, *B. americanus*, *B. regularis*, *etc.*). As with most elaborated poisonous secretions, the venom is a complex mixture of various cardioactive sterols having 6-membered lactone rings at C_{17} known as bufadienolides or bufagins, *e.g.*, bufalin (XV), bufotalin, telocinobufagin, bufotalidin, gamabufotalin, cinobufotalin, *etc.*); various cardioactive bufotoxins (*e.g.*, viridobufotoxin, vulgarobufotoxin, cinobufotoxin, gamabufotoxin, marinobufotoxin, *etc.*); various potent sympathomimetic catecholamines (*e.g.*, epinephrine, norepinephrine); various pressor, hypotensive, and smooth muscle-contracting indolealkylamines (*e.g.*, 5-hydroxy-dimethylaminotryptamine, serotonin, cinobufotenine, *etc.*); various noncardiotonic sterols (*e.g.*, cholesterol, provitamin D, γ-sitosterol, *etc.*); and various miscellaneous substances (*e.g.*, 51–61% water, mucoproteins, suberic acid, arginine, *etc.*).



XV

Some of the toad preparations, like the Chinese product, "ch'ansu," have been long employed in the treatment of canker sores, toothache, sinusitis, and local inflammations. This early and continued folklore use has prompted investigations on isolated principles and one which is readily available (cinobufagin) has been employed clinically. However, it appears to offer no advantage over ouabain due to its short action and side effects.

Despite evidence of this sort, bufomarine has been recommended for the treatment of heart ailments of the aged (388). Chen (389) feels that none of the toad cardiotonic principles can be justifiably employed in cardiology. However, continued studies on these cardioactive sterols, and the local anesthetic effects of some (*e.g.*, bufalin, cinobufotalin, and gamabufagin) may yet lead to useful drugs. Certainly the toad venoms yield an interesting array of some 30-odd pharmacologically active substances many of which were hitherto unknown.

Perhaps the most striking frog venom which has received much attention recently is that obtained from the Colombian kokoá frogs (*Phyllobates aurotaenia*). Interest in this venom dates back to the mid-19th century and naturalists have been long aware of its use by the Choló Indians of the Chocó jungle in western Colombia. The natives mimic the sound of frogs to locate and capture it, and thence handle it carefully with a large leaf (to avoid absorption of venom into minor cuts) prior to preparing the darts for dipping.

[3] Protopam, Ayerst Labs, New York, N. Y.

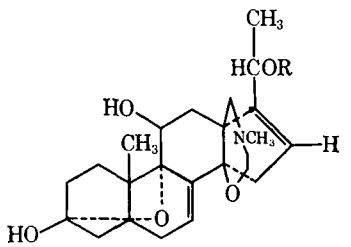
The frog is impaled alive on a stick, held over an open fire, and the oozing venom collected on the tips of the darts. Blowguns are used to propel the poisoned darts which produce paralysis, convulsions, and death within minutes after piercing the skin of the animals (390–392).

Because of the difficulty in obtaining sufficient quantities of venom, it has taken several years to isolate the active principles. Witkop *et al.* (392) working with venom collected on early expeditions had earlier succeeded in isolating three alkaloids designated as batrachotoxin and batrachotoxinin A and B.

Batrachotoxin is known as the most potent cardiotoxin, and the most potent neurotoxin among the venoms. The effective dose for a dog is less than 500 ng./kg. Hence the frog venom is from 5–10 times more active than the next most potent substance in a venom, tetrodotoxin.

Current research on milligram quantities of very unstable venom derived from thousands of poison dart frogs from the Colombian jungles has allowed the structural elucidation of one derivative which gives clues as to the actual formula of the major active component, batrachotoxin. The recent chromatographic separations show that batrachotoxin occurs with its isomer isobatrachotoxin and both show similar toxicity. A third component called pseudobatrachotoxin is present and co-chromatographs with the iso form. However, because the pseudo derivative is unstable, it readily converts to batrachotoxinin A in the presence of water (391).

The recent structural elucidation of batrachotoxinin A shows that it is an unusual steroidal alkaloid (XVI).



R = H

XVI

It is believed to be derived from the unstable pseudobatrachotoxin mentioned above. Although its toxicity is only about one five-hundredths of the original venom's potency, it is still as poisonous as strychinine.

High-resolution mass spectroscopy shows that the three isomers are closely related and have the same molecular formula $C_{24}H_{33}NO_4$. Because of the small amounts of material, crystal size, and position of bromine atom in the molecule, several difficulties were encountered in determining the structure by the classic heavy-atom technique. Hence, a new phase-determining formula for noncentrosymmetric crystals (the tangent formula) was employed which ultimately yielded the structure of batrachotoxinin A.

Not only is this an unusual alkaloid obtained from an animal source, but it is in addition a steroid which accommodates a precursor of choline. This combination was heretofore unknown. In addition, steroids are not usually so extremely active. Research is continuing on the preparation of crystalline derivatives whose structures can be determined. Already the structure of batrachotoxinin A could serve as an excellent model for a whole new series of cardioactive and neuroactive molecules potentially useful as drugs.

Interesting cytotoxic substances, hemolyzing proteins, and antibiotic peptides are described in skin secretions of unks (*Bombina* spp.), tree frogs (*Hylaarborea*), and European newts (*Triturus* spp.) (393, 394).

Reptiles—Like the amphibians, the reptiles form a separate class of vertebrates too. This group has in common a dry skin, covered with horny plates or scales. They are considered to have descended from the amphibians. The order Squamata, suborder Serpentes, contains the sea snakes, which are the ones of major concern here.

This group of true snakes are highly specialized and maintain an exclusively marine or aquatic life. They have a complete lack of limbs, sternum, ear openings, and urinary bladder, and their bodies are covered by scales. Further, the eyes are lidless, immobile, and covered by transparent scales. They have a protrusile, slender, forked tongue and the body is posteriorly compressed into a flattened, paddle-shaped tail. The venom fangs are reduced in size (cobra type) and the maxillary teeth serve as hollow fangs.

Sea snakes average 3 to 4 ft. in length but some may attain a length of over 9 ft. They primarily inhabit the tropical Pacific and Indian Oceans being found as residents of sheltered coastal waters and river mouths. However, some species may be found as far as 100–150 miles from land.

These snakes are very awkward on land but have demonstrated remarkable adaptability to water in that they have juxtaposed and hexagonal scales and outstanding ability to swim backward or forward. They are able to float for long periods of time and are even able to remain submerged for hours.

The food of the sea snakes is captured underwater, consisting entirely of fish which are swallowed headfirst. Particularly they feed on the ocean bottom around rocks and in crevices where they capture fish and eels by killing them with a vigorous bite of their venomous jaws.

For the most part, many species are docile, while a few are aggressive. The venom potency is considered to be about 50 times as potent as that of the king cobra. A few representative species include the sea snake, *Enhydrina schistosa;* the chittul or banded sea snake, *Hydrophis caerulescens;* the sea snake, *Hydrophis nigrocinctus;* and the yellow-bellied sea snake, *Pelamis platurus.* The last species is the most widely distributed and all of these are marine. The only fresh-water species known is *Hydrophis semperi* found in the fresh-water Lake Taal, Luzon, Philippine Islands.

The bite of the sea snake produces characteristic symptoms which develop slowly from 20 min. to several hours after envenomation. These include drooping eyelids, mild euphoria, anxiety, aching, dilated eyelids, sensations of tongue thickening, weak and irregular pulse, muscle stiffness, ascending paralysis beginning with the legs and moving up into the trunk, arms, and neck muscles. One of the outstanding characteristics is lockjaw, with speaking and swallowing becoming in-

creasingly difficult. Terminal symptoms include intensification of those signs given above with final cyanosis, clammy skin, convulsions, respiratory collapse, unconsciousness, and death. Mortality rate is about 20% (13).

According to various sources (3, 10), most of the 250 species of venomous snakes are found in four families including the Crotalidae, Viperidae, Elapidae, and Hydrophiidae. About 35,000 deaths per year have been attributed to envenomations by members of these families. The Hydrophiidae contain the sea snakes.

Most snake venoms are a complex mixture of proteins, the large majority of which possess potent enzymatic activity. The true lethal factors are usually nonenzymatic proteins of relatively low molecular weight. Many reports in the literature give the overall toxicity of the whole venom of many species of poisonous snakes, while few concentrate on the true lethal factors and their specific pharmacological effects (3).

Some of the important proteinaceous enzymes isolated from snake venoms include proteinases, transaminases, hyaluronidases, L-amino acid oxidase, phosphatidases, cholinesterases, anticholinesterases, lecithinase A, ribonuclease, deoxyribonuclease, phosphomonoesterase, phosphodiesterase, 5′-nucleotidase, ATPase, DPNase, and DPN-pyrophosphatase. Any one snake venom may contain five to 15 of these enzymes, about three to 12 nonenzymatic proteins, and polypeptides, and six or so other active substances. It is not difficult then, to appreciate the fact that most snake venoms have an effect on every organ system and perhaps on every cell (3).

The effects of whole snake venom are deleterious to the cardiovascular and blood systems, the respiratory system, and the nervous system. There are many obvious synergistic and antagonistic effects of the venom mixture. Overlapping effects make it difficult to ascertain which principles in a given venom exert which effects on the body. Much more study is needed to collect sufficient quantities of venom from specific poisonous species to obtain potentially new and useful pharmacologically active substances.

One example of active materials isolated from the sea snake venoms is seen in the recent crystallization of erabutoxins A and B from *Laticauda semifasciata* venom and of laticotoxin A from *Laticauda laticaudata* venom (395–399). The toxins are protein in nature, contain varying amounts of 61 amino acids, and have molecular weights from 6,760 to 7,430. Their LD_{50} values (mice i.m. injection) range from 0.15–0.13 mcg./g. body weight

Although relatively small amounts of crude venom (*e.g.*, 5 g. from 350 individuals) were obtained and considerable difficulties encountered in purification and crystallization (chromatography, disk electrophoresis, and ultracentrifugation), sufficient quantities of pure material were obtained to carry out careful quantitative pharmacological experiments. This enabled the determination that the lethal dose values of the three toxins are almost the same and are of the same order as cobratoxin, which was previously obtained in crystalline form from the Formosan cobra (*Naja naja atra*).

The erabutoxins were found to block neuromuscular transmission and also inhibit the contracture of frog rectus abdominis muscle by acetylcholine. These observations have suggested that the toxins act on the postsynaptic membrane in a manner very similar to curare.

Another neurotoxic component of the venom of the sea snake *Enhydrina schistosa* has been isolated by Cary (400). Thus, continued investigations on the sea snake venoms should lead to other useful pharmacologically active new molecules. Some of the specific enzymes in snake venoms may also prove useful in basic studies on cellular metabolism and pharmacological mechanisms could ultimately lead to discoveries of biomedical import.

The last group of reptiles of interest are the marine turtles. Toxicities here are due mainly to ingestion of certain species which may normally be eaten, but which may become toxic under certain circumstances. The species implicated are found in the vicinity of the Philippine Islands, Ceylon, and Indonesia. They include the green sea turtle (*Chelonia mydas*), the hawksbill turtle (*Eretmochelys imbricata*), and the leatherback turtle (*Dermochelys coriacea*).

Some of the symptoms develop within a few hours to several days after ingesting the flesh. These include nausea, vomiting, diarrhea, severe upper abdominal pain, dizziness, and dry burning sensation of the lips, tongue, and lining of the mouth and throat. These precede more severe symptoms, *e.g.*, severe oral and tongue lesions and ulcers, deep sleepiness, and ultimately liver and kidney damage leading to death. Some 44% of the victims poisoned by marine turtles die (13).

Practically nothing is known about the true mode of poisoning and the nature of the toxin(s). Certainly, more research is indicated in this area to determine the nature of the CNS- and gastrointestinal-active agents.

Mammals—This group of chordates encompasses an extremely large and broad range of animals. They are characterized by nourishing their young with milk, most have hair, all are warm-blooded, the heart four-chambered, and the body cavity divided by a diaphragm.

Relatively few of the mammals are implicated in poisonings of humans. These for the most part cause trouble on ingestion. Included here are the polar bear (*Thalarctos maritimus*), the bearded seal (*Erignathus barbatus*), the Australian sea lion (*Neophoca cinerea*), and certain species of whales and dolphins. The flesh and particularly the livers of these have caused a wide range of toxicities in man. However, very little is known about the nature of the poisonous principles here except that they are for the most part neuroactive and gastrointestinally active.

One very interesting mammal which is really aquatic and not marine which should be reinvestigated is the duck-billed platypus (*Ornithorhynchus anatinus*). Not only is it the only member in its family (Ornithorhynchidae) but it is the only one of two families of mammals that lay eggs.

It is limited to the southern and eastern parts of Australia and Tasmania. The animal excavates a burrow for itself in the banks of slow streams which it frequents. It feeds on animal foods, grubs, worms, snails, and mussels.

The platypus is covered with dense fur of blackish-brown color, its limbs are short, five-toed, and webbed, and the tail is longish and broad, being flattened from

above downwards. The "beak" is broad and flat and covered with a soft sensitive naked skin which abounds in sense organs of a tactile nature.

Perhaps of most interest is the little-known fact that the platypus is venomous. On the inner side of each hind limb of the male is a movable spur. This is found in the young female too; however, in the mature female it disappears leaving only a depression to mark the spot where it had been. The male spurs are situated over a cyst or crural gland of venomous fluid, and they possess a tube or cannula through their centers through which the animal can force the poison into a wound.

These strong crooked spurs are located at the setting of the heel and are 1.2 cm. (0.5 in.) long with a sharp point. Moving the point of the spur close to the leg conceals it in the hair of the animal. When it is directed outward, it projects conspicuously. In addition to serving as a set of curved caliper-like venomous pincers, they are used by the male platypus to prevent the female from withdrawing herself during the act of copulation.

Although the venom appears to vary in strength during different times of the year, perhaps in accordance with seasonal hormonal variations, there is little doubt as to its potency and effects.

Burrell (401) reviewed the anatomy, physiology, and histology of the gland, duct, and spur and shows clear photographs of the poison gland connected by duct to the well at the base of the spurs.

In addition, to several personal accounts of poisonings in man (intense pain, partial paralysis, intense swelling, subsiding after a week) and deaths in dogs, he gives the results of several experiments on the effect of the isolated venom on other animals. Briefly, in one experiment, 0.06 g. of venom injected through a cannula into the jugular vein of an anesthesized rabbit produced, within seconds, a precipitous blood-pressure drop of 40 mm. Hg, depressed and fewer heartbeats, hurried and exaggerated respiration, expiratory convulsions, and finally death within 90 sec. Immediate postmortem revealed clotted blood in the right side of the heart and the whole of the venous system. The left chambers of the heart and pulmonary veins were found to contain fluid blood and there was extensive endocardial hemmorrhage. It is noted that these effects resemble poisoning by i.v. injection of snake venoms although in the latter, the effects are some 5,000 times as virulent. The few chemical experiments conducted indicated that the platypus venom was proteinaceous in nature.

Once again we have here an interesting venom, albeit from an aquatic mammal, which warrants further investigation. There is no reason to doubt that this venom may yield active substances much like the snake venoms discussed earlier. If nothing else, a study of the constitution of the venom and relating it to reptile venoms may yield further evolutionary evidence of the proximity of this primitive, egg-laying mammal to the reptiles from which it is purported to have evolved.

CHEMISTRY AND PHARMACOLOGY

By now it is obvious that many different chemical classes of pharmacologically active substances are found in marine organisms. These include highly active and toxic proteins and polypeptides in many venoms; polyfunctional amines, *e.g.*, serotonin, tetramine, octopamine, tyramine, and histamine; novel and different amino acids, *e.g.*, kainic acid, domoic acid, GABA, taurine, and laminine; quaternary compounds, *e.g.*, homarine, pahutoxin, and betaine; enzymes, *e.g.*, amine oxidases, sulfatases, thiaminases, and phospholipases; nucleic acid derivatives, *e.g.*, spongouridine and spongothymidine; polysaccharides, *e.g.*, carrageenan and laminarin; vitamins, *e.g.*, thiamine and cyanocobolamin; aromatic bromo compounds, *e.g.*, aplysin, aplysinol, and 2,6-dibromophenol; fatty and other acids, *e.g.*, morrhuic acid and acrylic acid; sterols, *e.g.*, fucosterol and cholestanol; steroidal glycosides, *e.g.*, holothurin A; saponins, *e.g.*, asterosaponin A and B; terpenoids, *e.g.*, crassin; an amino perhydroquinazoline, *e.g.*, tetrodotoxin; a choline derivative, *e.g.*, murexine; guanidine-type compounds, *e.g.*, dinoflagellate toxin; an amine disulfide, *e.g.*, nereistoxin; and many others which have been alluded to in the foregoing discussions.

Generally, it may be stated that the majority of the relatively low molecular weight, easily identifiable molecules with biological activity (serotonin, homarine, *etc.*) have been pretty well characterized wherever they have been encountered in marine organisms. Those of intermediate molecular weight and moderate complexity (pahutoxin, aplysin, *etc.*) offer more resistance to characterization but are being elucidated slowly as interest develops and material becomes available to researchers. The higher molecular weight compounds with fairly complex structures (tetrodotoxin, holothurin A, *etc.*) offer great challenges to the skill of organic chemists and have recently occupied the entire efforts of competing teams of synthetic and natural product chemists around the world.

The highly active toxic proteins and polypeptides remain the greatest challenge in structure elucidation and perhaps a decade awaits the development of these into useful drugs. However, this last group may prove to be the most important because of their great specificity and apparent lack of side effects.

Recent reviews on the chemistry of marine-derived biodynamic agents include those by Nigrelli (2, 16), Russell (3), Halstead (4), Scheuer (402), Courville (403), Kaiser (404), Schwimmer and Schwimmer (8), and Barme (405). For the most part these reviews discuss the various compounds isolated using a biological classification. This is due mainly to the lack of sufficient chemical data to consider the active principles in any other fashion. In many cases only a relatively few physical characteristics (*e.g.*, pH, specific rotation, *etc.*) have been determined because of the crude nature of the material being worked with or its relative instability. Pharmacological tests generally are used to determine quality and quantity of activity and which fractions of mixtures contain activity.

Where possible, chromatographic and/or ion-exchange methods are used to determine uniformity and purity of isolated materials. In some cases carbon and hydrogen analyses yield molecular weight, which, coupled with other analytical data (UV, IR, NMR, *etc.*), help ultimately in structure characterization.

Many reports indicate that various investigations are at different stages of completion depending on amount

of biological material originally available, relative stability of material, continuity maintained by various research teams on given projects, and complexity and difficulty of chemical structure elucidation and separation problems encountered.

It may be stated with a fair amount of confidence that, given the necessary time and effort, the chemical structure of practically all biologically active materials from marine organisms could be determined by currently available methods. The major difficulties still lie in material procurement and the marshalling of the necessary multidisciplinary assault on the particular problem.

With respect to generalizations about pharmacological activity, one can see from Table I that effects on practically all physiological systems have been noted, *viz.*, CNS, respiratory, neuromuscular, autonomic, cardiovascular, and gastrointestinal, including various local effects. A recent review by Lane (406) on toxins of marine origin considers the highlights of pharmacological studies on various organisms. Included in the review are the most virulent toxins known with some consideration of their mode of action at the cellular level which has helped clarify some normal metabolic functional processes in cells and tissues.

According to Russell (26) marine toxins as a whole are much more varied in their chemical composition than their terrestrial counterparts. However, a degree of consistency is seen within a particular phylum indicating certain biogenetic relationships. Simpler marine forms tend to have single or few components in their poisons while higher forms tend to have more complex poisons.

Pharmacologically, the effects of marine toxins vary as dramatically as their chemical properties. Certain toxins have simple effects like transient vasodilation or vasoconstriction while others can effect more complex responses, *e.g.*, parasympathetic or sympathetic dysfunction or multiple concomitant changes in blood-vascular dynamics (26). To further cloud the issue, certain marine organisms may produce autopharmacological substances which frequently complicate the poisoning.

As to be expected when dealing with biological organisms there are innate qualitative and quantitative differences in the composition of toxins which cause variation from species to species and even from individuals within a species.

It is not difficult to see then how complications in solving problems here can be multiplied when the variations above are superimposed on differences in methods of extracting materials, differences in storage procedures and differences in separation, characterization, and testing methodology.

A basic understanding of variation in potency of various biologically active venoms on different organisms rests on the consideration that not all organisms evolved side by side and at the same time. As pointed out by Russell (26), for example, the venom of the black widow spider did not evolve and adapt to the problems existing between that spider and mammals. Hence, one can rationalize why its venom is 20 times less lethal to some arthropods than it is to the mouse. Further, the venom is also 10 times more lethal to certain other arthropods which did not adapt in the same fashion.

In an analogous manner some sharks appear to be relatively immune to stingray venom while others from different habitats are extremely sensitive to the toxin. Certainly, this phenomenon must be kept in mind when using various animals for testing purposes. Put in another way, species variation is a very critical consideration in the pharmacological evaluation of biologically produced toxins and venoms.

Notwithstanding all of the problems considered above, a sufficient number of pharmacologically important phenomena have been noted to warrant further research on newer screening procedures to detect different and unusual activity possessed by different marine organisms as well as basic biochemical studies on their mechanisms of action.

CURRENT RESEARCH

The unusual nature and variety of marine organisms has also prompted much basic biomedical research. Some of the studies in different laboratories include the research on hagfish heart (it has three) where some principle performs the pacemaking function and also initiates heart beat in several other kinds of animals; skin-graft research on hagfish which has no thymus and therefore, no immune defense system to reject grafts; genetic studies on the parthenogenic molly fish; the potential use of sea cucumber-type nerve toxins which are capable of "freezing" nerves without damaging them, for possible use in postoperative treatment of amputations, teratological studies using sea urchins which by virtue of their relatively simple life cycles, show deleterious effects of injected substances in days instead of weeks or months, studies of mussels for clues to multiple sclerosis; and even research on the nature of barnacle cement for possible use in dentistry to glue fillings in teeth, *etc.* (30).

There are many other projects of similar nature including re-evaluation of the folklore use of sea products as drugs in many countries, but space does not permit their full consideration.

As stated by Halstead (18) over five years ago, "There is need today in industry for a serious re-evaluation of what natural products research has to offer in the search for new products. More productive exploitation of this field is dependent upon improved procurement methods, more effective means of preserving crude botanicals and their phytochemical constituents in the field and more thorough pharmacological screening technics than now exist. Most disappointments in natural products research stem largely from our own technological failures to meet the challange of the field."

Interest is slowly gathering in the area of the development of marine pharmaceuticals and Senator Warren G. Magnuson (D.-Wash.) has recently stated a case for and proposed a bill (S.2661) to create a National Institute of Marine Medicine and Pharmacology as part of the National Institutes of Health (407). The establishment of such an institute would go a long way in promoting needed research in this field.

It is hoped that this modest survey will bring to the attention of all researchers, particularly those in the pharmaceutical sciences, the current status of marine biochemical and pharmacological studies and the need

for continued work in an area which holds much promise in alleviating the diseases of mankind.

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RESEARCH ARTICLES

Influence of the State of Molecular Aggregation on the Enzymic Hydrolysis of Arylsulfate Esters

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Abstract □ While the acid-catalyzed hydrolysis of potassium dichloronaphthyl sulfate is considerably enhanced when the substrate is bound to the surface of a micelle-forming surfactant, the enzyme catalyzed hydrolysis of this substrate exhibits a marked overall inhibition when the substrate is bound to the same surfactant. A plot of the rate of enzymic hydrolysis of that fraction of the substrate bound to the surfactant micelle *versus* surfactant concentration exhibits a sharp maximum. It is postulated that this maximum can be attributed to a gross change in the composition or structure of the surfactant-substrate micelle, such that the required "fit" of the bound substrate on the enzyme surface is sterically blocked. This "critical" surfactant concentration is also evidenced by an abrupt change in the extent of substrate-surfactant interaction under similar conditions. The rate of hydronium ion-catalyzed hydrolysis of potassium dichloronaphthyl sulfate bound to the same surfactant exhibits no such maximum.

Keyphrases □ Arylsulfate esters—synthesis, enzymic hydrolysis □ Enzymic hydrolysis—molecular aggregation effect □ Critical micelle concentration—conductivity measurements □ Surfactants, micellar—arylsulfate binding □ UV spectrophotometry—analysis

The micelle-forming nature of many compounds of biochemical interest and the involvement of some of these compounds in biologically important reactions suggest that there may be similarities between chemical reactions occurring in micellar systems and enzymic reactions occurring when the substrate is in micellar solution or bound to a macromolecule.

It has been known for a considerable time (1, 2) that the addition of bile salts increases the rate of hydrolysis of fat by pancreatic lipase. The mechanism by which