Antimicrobial Activity of Certain Marine Fauna. (25839)

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The inhibitory effect of canned abalone juice on experimental poliomyelitis in mice and that of frozen fresh abalone juice on *Staphylococcus aureus in vitro* has been described(1). Following that discovery a number of sea animals were tested as possible source of antimicrobial agents. These included oysters (*Ostrea virginica*), clams, (*Venus mercenaris*), lobsters (*Homaridae americanus*), shrimps (*Crago vulgaris*), flounders (*Pseudo-pluronectes americanus*), butter fish (*Poronotus triacanthus*), and crab (*Callinectes sapidus*). *Abalone* (*Haliotis refescens*) was also included for positive control. The results are here reported.

Materials and methods. Fresh live oysters, clams, and lobsters; and fresh shrimps, flounders and butter fish kept on ice were purchased in the sea food market. Cooked crab meat was purchased in the food market. Abalone was obtained from California in frozen state.* A penicillin sensitive strain, FDA 209, and a penicillin resistant strain of *Staphylococcus aureus* and a strain of *Bacillus subtilis* were used as test organisms.† *Preparation of extract.* Oysters, clams and tails of lobsters without shells, shrimp without heads and shells, flounders and butter fish without viscera and white crab meat were ground separately in meat grinder. Ground tissue was heated in container with equal weight of water at 90-95°C for 30-45 minutes. Heated material was centrifuged at 1,500 rpm for 15 minutes and the supernatant fluid was kept at −20°C before assay. Viscera with their contents, of abalone, lobsters, flounders and butterfish were also prepared as described above. The viscous grayish-white fluid left in container for oysters was regarded as oyster juice. A brownish fluid obtained from frozen fresh whole abalone upon thawing was regarded as abalone juice. These juices were heated to 70°C for 30 minutes and centrifuged at 1,500 rpm for 15 minutes before assay. No extract of abalone meat was tested in the present study. The pH of all extracts or juice was adjusted between 6.9 and 7.1 before assay. *Method of assay.* This was intended only for screening accuracy. Three consecutive 24-hour transfers of *Staphylococcus aureus,* FDA 209 strain, were made prior to using the culture as inoculum. Difco nutrient medium at pH 7.0 was used for all transfers and for assay. Ten-fold dilutions of 24-hour culture were made in buffered saline and a loopful (5 mm in diameter) of each dilution was inoculated into a Wassermann tube containing 1.8 ml of nutrient broth with 0.2 ml of the extract or juice to be tested. Corresponding dilutions of the same test organisms were also inoculated into a series of tubes with 2.0 ml of broth alone. After incubating at 35°C for 18 hours, a loopful from each tube was spread on a quarter of a nutrient agar plate which was then incubated at 35°C overnight and colonies of growth thereon were counted. Absorbent paper disc method for assay of antibiotics was occasionally used. Paper discs impregnated with test material were placed on surface of agar plates upon which a 24-hour broth culture had previously been spread. After overnight incubation a clear zone around the disc indicated inhibition.

Results. Abalone juice, oyster juice and extract and clam extract all demonstrated antimicrobial activity while all others were apparently negative as revealed by fluid method of assay (Table I). This method was used instead of conventional absorbent paper disc method, because the latter gave only negative or inconsistent positive results for abalone juice, as mentioned previously(1). However, in the present study when the penicillin sensitive strain of *Staphylococcus aureus* was used as a test organism, clam extract showed rather

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TABLE I. Effect of Juice or Extract of Various Sea Animals on Growth of *Staphylococcus aureus*, FDA 209 Strain.

Juice or extract of sea animals		Colonial growth from 1 loopful of 18 hr broth cultures inoculated with serial dilutions							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	Control
Abalone juice	+	0	0	0	0	0	0	0	0
	—	Con	Con	Con	Con	0	0	0	0
Oyster "	+	9	4	0	0	0	0	0	0
	—	Con	Con	Con	Con	TN	0	0	0
Oyster extract	+	105	0	0	0	0	0	0	0
	—	Con	Con	Con	Con	Con	0	0	0
Clam "	+	0	0	0	0	0	0	0	0
	—	Con	Con	Con	Con	Con	0	0	0
Lobster "	+	Con	Con	Con	TN	TN	TN	0	0
	—	"	"	"	Con	Con	0	0	0

Control: Broth with test material but not inoculated.
9, 4, etc.: Indicating No. of colonies.
TN: Colonies too numerous to be counted but not confluent.
Con: Confluent growth.
+ = present; — = absent.
Results of crab, flounder, shrimp and butter fish are similar to that of lobster.

consistently a 3 mm inhibiting zone around the disc while oyster extract was negative. By the fluid method of assay, clam extract also demonstrated moderate inhibitive action against a penicillin resistant strain of *Staphylococcus aureus*.

Extracts of viscera of some of these sea animals did not show definite or marked inhibitory effect. However, on agar plates inoculated with a broth culture containing viscera extract of abalone, colonies of *Staphylococcus aureus* were not only much less numerous but also much smaller in size than those in the controls, indicating some inhibition. In separate studies‡ now in progress, it is shown that, after chemical treatment, abalone juice and abalone viscera extract both possess marked antimicrobial activity against penicillin sensitive and penicillin resistant *Staphylococcus aureus* and *Bacillus subtilis* in a concentration as low as 1.0 mg %.

Discussion. These experiments extend our previous study on antimicrobial activity of abalone juice. The fact that an inhibitory effect was demonstrated much more easily in fluid medium than on solid agar suggested that the active agent was not readily diffusible in the latter medium. The active agent was heat stable, and non-dialyzable(1). Its properties are not typical of any known antibiotics(2,3) which are usually readily diffusible on solid agar medium, heat labile and dialyzable. It was not determined whether the action was bactericidal or bacteriostatic.

Whether the agent was located in viscera contents or in body tissue, and whether there were more than one agent cannot now be stated. It is possible that some antimicrobial agent or agents present in the aquatic environment are ingested by sea animals, undergo chemical changes *in vivo*, and result in a modified but active antimicrobial compound or compounds. The antimicrobial effect of some of the agents concerned can only be demonstrated when they are freed from accompanying nutritious substances and growth factors. Further work along such lines including *in vivo* experiments and virus studies is in progress.

Summary. A limited number of sea animals were screened for antimicrobial activity against *Staphylococcus aureus*. In addition to abalone juice, whose antimicrobial activity had been reported, extracts of oysters and clams possessed marked inhibitive effect. Substance of this nature may be valuable therapeutic and prophylactic tools.

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‡ Chemical purification is being carried out by Dr. B. Prescott, Nat. Inst. of Allergy and Infect. Diseases.

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Time Study of Acute Cold-Induced Acceleration of Thyroidal I^{131} Release in the Hamster.* (25840)

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Previous experiments(1,2) have shown that rate of hormone release from hamster thyroid is accelerated markedly during first 12 hours exposure to cold (5-6°C). The data indicated that this acute release of thyroidal I^{131} is dependent upon a neural mechanism which discharges TSH from the pituitary gland. The present study is a more careful analysis of some hypothalamo-pituitary-thyroid relationships during this initial 12 hour period of cold.

Methods. Adult hamsters of both sexes weighing 90-110 g were used. Twelve to 18 hours after intraperitoneal injection of 4-8 μc of I^{131}, thyroidal uptake was measured by counting externally neck and hind-leg regions with shielded scintillation crystal. The neck-minus leg counts, expressed as per cent of injected dose, represented thyroid uptake. The fractional rate of thyroidal I^{131} release was estimated by subsequent daily measurement of thyroid activity, each day's calculation being corrected for physical decay and expressed as percentage of initial 12 hour uptake. After at least 3 days of external counting to determine normal slope of release curve and immediately after zero count at room temperature (20-22°C), groups of hamsters were exposed to cold (5-6°) for following periods: 0.5, 1, 2, 3, 4, 6, 8 and 12 hours. During periods of cold exposure, animals were housed individually in metal cages with minimum of bedding; free access to food and water was permitted. After desired period of cold, thyroid activity was again measured externally, blood samples taken for determination of PBI^{127} and thyroid glands removed and weighed. Radioactivity was measured on one excised lobe, then prepared for determination of total iodine content. Posterior lobes of pituitary glands were separated by blunt dissection and adenohypophyses of at least 8 animals of each group were pooled, homogenized in acidified saline and frozen until assayed for thyrotropin (TSH) content. A time-study of response of hamster thyroid to exogenously administered TSH was carried out in same fashion as the time-study of thyroid response to cold. After zero external count, not less than 10 animals/group were injected intraperitoneally with one unit of TSH (Armour Thtropar). Ability of thyroxine to block cold-induced release of thyroidal I^{131} was examined. Freshly dissolved L-thyroxine was administered subcutaneously to groups of not less than 8 animals in doses ranging from 2.5 to 20 μg at varying times before and after beginning of 12 hours of cold exposure.

Results. Response to cold. To express quantitatively the accelerated release produced by varying periods of cold exposure, normal (room temperature) release curves of each animal were continued, appropriate number of hours (corresponding to number of hours of cold) to determine a theoretical position of the curve at room temperature. The difference between this point and the actual position of release curve determined after cold exposure is considered per cent of accelerated release. Fig. 1 summarizes time sequence of accelerated release from hamster thyroid at-

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