CCXXII. EMULSIN IN CERTAIN MARINE IN- VERTEBRATES AND MICRO-ORGANISMS.

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(Received July 31st, 1934.)

ACCORDING to Giaja [1906] emulsin had not been reported in marine animals prior to the time of his researches on the subject. He investigated a number of molluscs, demonstrating amygdaloclastic emulsin in the intestinal tracts of the gastropods: *Aplysia punctata*, *Patella vulgata*, *Trochus turbinata*, *Buccinum undatum*, *Doris tuberculata* and *Haliotis tuberculata*; in the lamellibranchs: *Tapes desiccata*, *Pecten maximus*, *Mya arenaria* and *Mytilus edulis*. Neither the cephalopods nor any of the fishes that he examined showed the presence of emulsin, whereas the echinoderms *Asterias glacialis* and *Echinus acutus* were shown to possess it.

Later investigations by the same author [1907] showed that the alimentary tract of several genera of marine crustacea and one fresh-water genus possessed the ability to hydrolyse amygdalin and a number of other glucosides. Bierry and Giaja [1906] had demonstrated the presence of emulsin and lactase in the land gastropods *Helix*, *Limax*, *Lymnaea* and *Planorbis*, and Giaja [1910] carried out further studies on the capacity of the digestive juice of the edible snail, *Helix pomatia*, to hydrolyse amygdalin with the production of hydrocyanic acid.

While it has long been known [Buchanan and Fulmer, 1930] that many terrestrial, fresh-water and intestinal bacteria, as well as a number of yeasts and moulds, are able to utilise amygdalin in their nutrition, some having been shown to do so with the production of hydrogen cyanide, it has not been previously recorded, to the writer's knowledge, that many marine bacteria and kindred micro-organisms possess this property in sea-water nutritive media containing amygdalin.

The desirability of investigating a number of micro-organisms with regard to this property was brought out especially with reference to the possibility of their playing some rôle in digestive processes in the alimentary tracts of marine animals.

For the detection of HCN Guignard's test [see Haas and Hill, 1932] and the silver iodide test developed in this laboratory [Fox, 1934] were used. For quantitative determination of HCN produced, the aeration method used by Roe [1924] and by Bishop [1927] was employed.

Toluene-preserved aqueous extracts of either the whole alimentary tract or of the crystalline style alone¹ of the large California sea mussel, *Mytilus californianus*, produced free HCN from amygdalin substrates at room and at blood temperatures. Heating to boiling completely inactivated the enzyme. Living

¹ Yonge [1926] demonstrated an amygdaloclastic enzyme in the digestive diverticulum of the oyster, *Ostrea edulis*, but detected no such enzyme in the style of this mollusc.

mussels (two) kept in an aerated solution of 0.1 % amygdalin in sea-water produced in 43 hours about eight times as much HCN as did naturally-occurring mixed marine micro-organisms in the same volume of sea-water in the absence of the animals (*i.e.* 0.00044 as contrasted with 0.000054 g. HCN respectively). There was always a lag in the inception of amygdaloclastic action by mussel-emulsin in both the living and killed animals. In the presence of amygdalin, living mussels excreted the enzyme into the surrounding water. Here it retained its capacity to hydrolyse amygdalin after the removal of the animals and the preservation of the solution with toluene. In no case did amygdaloclastic micro-organisms, killed with this preservative or with chloroform, produce HCN from the glucoside.

Sixty-nine different species of bacteria, six actinomyces, three moulds and two yeasts (all isolated from the ocean in Dr C. E. ZoBell's laboratory, as yet unidentified as to species) were investigated.

(It should be stated here that the existence of typical marine moulds and actinomyces is still a moot question among bacteriologists and mycologists, but certain micro-organisms, indistinguishable from these respective groups, were isolated from the ocean here and will be correspondingly referred to in this paper.)

The *yeasts* and *moulds* were inoculated in duplicate from pure cultures on to sea-water agar slants containing nutritive materials and 0.1 % amygdalin¹. Controls of (1) each fungus without amygdalin in otherwise identical culture medium and (2) sterile amygdalin-containing culture slants were set up in each case. The tubes were incubated at 30°. In from four to ten days, HCN was produced by these fungi, excepting one of the two yeasts. Controls of both types were negative.

The six *actinomyces* were inoculated in duplicate from pure cultures into nutrient sea-water broth containing 0.1 % amygdalin, with the two kinds of parallel controls described above. The cultures were kept in the laboratory in diffused light, at room temperature, for 96 days.

While all of them multiplied rapidly and all eventually produced HCN in both tubes (all controls remaining negative) the interval preceding cyanogenesis was usually a long one (10–30 days).

Fig. 1. Numbers of isolated marine bacteria producing HCN from amygdalin, plotted against the passage of time in days.

Each culture of *bacteria* was prepared in duplicate in the same kind of sea-water medium as were the actinomyces and was maintained under identical conditions, with similar controls. The curve (Fig. 1) shows the course of HCN

¹ Coleman and Bell, C.P.

production by increasing numbers of cultures up to a total of forty-six with the passage of time in days.

The control cultures in all the experiments with micro-organisms (*i.e.* tubes containing organisms without amygdalin and those containing sterile solutions of the amygdalin) produced no HCN during the entire experiment.

The virtually complete agreement of both duplicates (only one exception in the case of a very late HCN-producer) and the fact that almost all cases of HCN production manifested themselves simultaneously in both duplicates, added to the fact that the amygdalin control tubes remained sterile, would lead one to conclude safely that all or the great majority of the cultures remained free from contaminants.

The tardiness of HCN production in a number of duplicate amygdalin cultures might indicate several interesting possibilities. One that has been suggested is that certain associated organisms of the dormant commensal type may spring up when the other strain has completed all or a part of its life cycle, and that these organisms differ from the others in physiological characteristics; another is the conceivable acquisition of new physiological properties with gradual adaptation to slowly increasing osmotic pressures attending the evaporation of water from the medium through the cotton plugs; and still another is the changing of metabolic habits with age, or with certain stages in the life cycle.

The experiments demonstrate that the California mussel can produce free HCN from amygdalin independently of the presence of micro-organisms and that the majority of marine bacteria and kindred micro-organisms (such of these as are capable of living and multiplying in sea-water) that were examined are capable of producing free hydrocyanic acid by destruction of amygdalin.

It was demonstrated by Emerson *et al.* [1913], and by Clawson and Young [1913], that pure cultures of *B. fluorescens*, *B. violaceus*, several strains of *B. pyocyaneus* and other bacteria described, but not named, produce HCN from a number of different protein substrates.

While the micro-organisms studied in this laboratory did not produce HCN from broth in the absence of amygdalin, and while certain forms produced it from the glucoside rather promptly, it cannot be stated with certainty whether HCN production was primarily the result of hydrolysis or of oxidation.

Since this physiological property is possessed by so many of the marine micro-organisms and by a number of herbivorous invertebrates, it seemed to be of interest to examine some plants of the ocean for the presence of amygdalin. This has been done without a single positive result.

Flourishing cultures of each of the yeasts, moulds, actinomyces and bacteria, when killed with heat, toluene or chloroform, failed to evolve HCN after the addition of a highly active emulsin, even after a long period of contact. Macerated leaves of the common eel-grass, *Zostera marina*, and crushed blades of the giant kelp, *Macrocystis*, and of the "feather-boa," *Egregia*, also gave negative results.

The capacity of living marine organisms to break down amygdalin and live in the presence of free HCN is interesting, but the detection of amygdalin or other cyanogenetic glucosides in marine plants which might serve as food for such organisms would render the above fact of considerable significance regarding certain aspects of nutrition and a part of the nitrogen cycle of the sea.

SUMMARY.

1. Living mussels (*Mytilus californianus*), or toluene-preserved extracts of either their viscera or the crystalline style alone, can bring about the hydrolysis of amygdalin with the production of free hydrocyanic acid.
2. In the presence of amygdalin, the mussel discharges into the water the enzyme which breaks down the glucoside.
3. Three marine moulds, one of two marine yeasts, six actinomycetes from the ocean and forty-six out of sixty-nine bacteria from the same source produced hydrocyanic acid from amygdalin after intervals of various durations.
4. None of the amygdaloclastic micro-organisms retained the cyanogenetic property when killed with toluene or chloroform.
5. None of the micro-organisms produced HCN from the peptone control cultures.
6. No source of amygdalin in marine plants, including all the micro-organisms studied, a marine flowering plant and some kelps, has so far been detected in this laboratory.

It is a pleasure to acknowledge the aid of Dr C. E. ZoBell, Mrs C. B. Feltham and Mrs T. Wells, of the Section of Microbiology of this Institution, for supplying me with cultures of the different marine micro-organisms studied and for giving helpful advice.

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