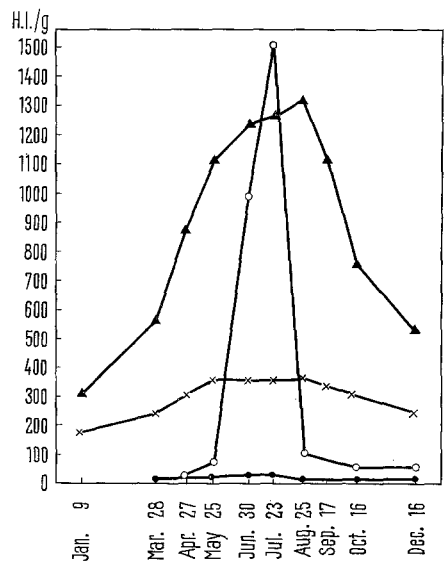
Distribution and Seasonal Variation of Toxic Principles of Sea-Cucumber (*Holothuria leucospilota* Brandt)

Naturally occurring saponins had been found only in plants until toxic principles were obtained from special Cuvier's glands and body walls of some species of sea-cucumbers and identified as saponins (holothurin A) by YAMANOUCHI[1] and NIGRELLI et al.[2]. Several other animal saponins were isolated in crystalline form[3,4]. All these were found in Echinodermata and designated holothurin B (from sea-cucumber) and asterosaponin A and B (from starfish). We report in this communication about the remarkable seasonal variation of such toxic principles and their distribution in the organs and body of the sea-cucumbers.

Toxic sea-cucumbers, *Holothuria leucospilota* Brandt (in Japanese, nise-kuro-namako), a species commonly found in Japanese warm waters, were collected along the coast of Wakayama, and separated into several organs and weighed. Each organ was extracted with ethanol at room temperature and filtered. The residue was extracted 3 times with 70% warm ethanol, the combined filtrate was concentrated under reduced pressure, defatted with ether and the aqueous layer was shaken 3 times with *n*-butanol, combined butanolic extract solution was concentrated under reduced pressure and the residue was used as samples. The amount of saponins in each organ is expressed by the hemolytic index (HI) per gram of wet organ on red blood cell from rabbit, and was determined according to the method of FUJITA and NISHIMOTO[5]. Standard Merck saponin used in control experiments showed HI of 33,000 on average. As shown in the Table, saponin was found in every part of the body of the sea-cucumbers, but ovaries and Cuvier's glands separated from the sea-cucumbers collected in July are particularly rich in saponin. The remarkably high concentration of saponin found in Cuvier's glands, the natural defence organ against predators, is easily understood from its function, but it is interesting to know that ovaries (in July) also contain an extraordinarily large amount of saponin whilst ovaries (in December) and testes (in December and July) are low in content. On the other hand, the saponin content of the organs in the animal body collected in July was always higher than that of the corresponding organs in December. Furthermore, seasonal variations of the saponins in various parts of the body, namely Cuvier's glands, body walls (epidermis and longitudinal retractor muscles) and gonads, were investigated almost monthly throughout the year of 1967. These data are shown in the Figure. It reveals that the amount of saponin in the ovaries containing mature eggs (June to August) reaches the highest value, 1500 HI/g, and then decreases rapidly after spawning (in September). It also indicates that saponin content in Cuvier's glands and body walls, rises from spring towards summer season and reaches a peak in June to August, the breeding season, and decreases during winter. Similar seasonal variation of the saponin content in starfish integument has been recognized by YASUMOTO et al.[6]. The physiological role of these saponins in Echinodermata is not known.

Distribution of toxin in the organs

Organs	HI/g in July	HI/g in December
Digestive organs	65	37
Longitudinal retractor muscles	282	262
Epidermis	430	286
Intestinal hemal vessels	666	430
Cuvier's glands	1250	526
Ovaries	1504	61
Testes	31	15



Seasonal variation of toxin in several organs. ▲, Cuvier's glands; ×, body walls; ○, ovaries; ●, testes.

Zusammenfassung. Die jahreszeitlichen Schwankungen des Saponingehaltes der Seegurke *Holothuria leucospilota* Brandt wurde untersucht. Hohe Saponinkonzentrationen fanden sich besonders in Cuviers-Drüsen und in Ovarien mit Eiern. Höchste Saponingehalte werden von Juni bis August gemessen.

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