The Variability in the Holothurin of Philippine Holothurians

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ABSTRACT

Thirty Philippine holothurians mostly from San Fernando, La Union and Calatagan, Batangas were investigated for their holothurin content and hemolytic activity. The ethanolic extraction and subsequent hemolytic assay on the extracts demonstrated variability in yield and potency. In yield: gut > Cuvierian tubules > body wall; body wall holothurin, Stichopus > Actinopyga = Bohadschia = Holothuria; *gut holothurin, no significant differences among genera; Cuvierian tubule holothurin,* Holothuria > Bohadschia. *In hemolytic potency: Cuvierian tubule > gut > body wall; body wall holothurin, Holothuriidae > Stichopodidae > Synaptidae = Chiridotidae; gut holothurin,* Bohadschia > Actinopyga > *Holothuria* > Stichopus; *Cuvierian tubule holothurin,* Bohadschia > Holothuria. *Time and locality of collection were insignificant factors for variation.*

INTRODUCTION

The toxicity of holothurin has been attributed in part to its surface activity, i.e., its ability to abstract membrane lipids, specifically, cholesterol. This action was most significantly exhibited by its hemolytic effects (Yamanouchi, 1955; Nigrelli, 1952; Jakowska *et al.*, 1958; Nigrelli, 1960; Nigrelli and Jakowska, 1960; Thron, 1964; Pocsidio, 1983 and 1988). It had been demonstrated that sea cucumber glycosides at below a critical micelle concentration disturbed the linear relationship in multilamellar liposomes composed of egg phosphatidyl

choline, phosphatidic acid and cholesterol but did not affect cholesterol-free liposomes (Yu and Jo, 1984). Thron in 1964 calculated a high "apparent intrinsic activity" for hemolysis of Holothurin A in that one molecule of the saponin could displace a cell membrane surface area of 8700A as compared to that of a commercial saponin, digitonin, with an "apparent intrinsic activity" of 1050A or that about 100 + 20 cholesterol molecules combine with each molecule of Holothurin A as contrasted to about only 12 + 1 for each molecule of digitonin.

Thus, the interaction of holothurin with red blood cells may render inadvisable its possible use as drug when administered intravascularly. It is believed that holothurin is the most potent animal saponin hemolysin. But there are still other potentials of the drug which are worth looking into. The use of the heolytic assay expedites the determination of occurrences of holothurin in different sea cucumbers.

The present report is a continuation of an earlier study on the hemolytic activities of holothurin (Pocsidio, 1988). This gives the hemolytic data obtained from an increased sample size. The variability in the hemolytic potency among species, genera and families of the holothurians in their holothurins from different structures and in relation to time and locality of collection of the animals as analyzed statistically indicate the representative distribution of the saponin in Philippine holothurians. It has become much more evident, however, that differences in the activities of the several sea cucumber glycosides can only be definitely deduced from experiments using the purified substances.

MATERIALS AND METHODS

The collection of specimens, processing of sea cucumbers for extraction of crude holothurin, ethanolic extraction, the hemolytic assay, and statistical analysis have been previously described (Pocsidio, 1988). Sea cucumbers were collected from several localities, mostly from San Fernando, La Union and Calatagan, Batangas. From the littoral areas were gathered the following species: *Actinopyga echinites, A. mauritiana, A. miliaris, Actinopyga* sp., *Bohadschia argus, B. graeffei, B. marmorata, B. vitiensis, Holothuria atra, H. coluber, H. fuscocinerea, H. hilla, H. impatiens, H. klunzingeri, H. nobilis, H. pervicax, H. pulla, H. rigida, H. sanguinolenta, H. scabra, H. tigris* of Family Holothuriidae, *Stichopus naso, S. chloronotus, S. variegatus, S. variegatus* var. *hermanii, Stichopus* sp., Family Stichopodidae, *Opheodesoma grisea, Pendekaplectana nigra,*

Synapta maculata, Family Synaptidae and *Polycheira rufescens,* Family Chiridotidae.

The hemolytic assay method after Fujita and Nishimoto (1952) was done on the remaining 34 samples of body wall, 11 gut and 1 Cuvierian tubule crude holothurins for a total of 72 samples of body wall, 32 gut, and 11 Cuvierian tubule extracts. Listed in Tables 1-3 are the samples, dates, and places of collection. While many of the specimens were collected from December 1983 to March 1985, the hemolytic tests on them were performed from January to August 1988. The extracts which were obtained almost immediately after collection had been stored in air tight jars kept inside a refrigerator.

RESULTS AND DISCUSSION

The data on the crude holothurin yield of the different parts of the sea cucumbers and their corresponding hemolytic activity are shown in Tables 1-3. A graphic representation of the data which emphasizes varying patterns based on mean values is Fig. 1. In this figure, the scientific names of different species were mainly abbreviated to their initial letters.

Statistical analysis had been done on the data of yield (Pocsidio, 1989). This gave on dry weight basis, crude holothurin yield ranging from 0.17% to 22.6% with highest content in the gut. In decreasing order of crude holothurin content, gut > Cuvierian tubules > body wall. The average percentages crude holothurin in these organs were for the gut 6.64%, Cuvierian tubules 4.28%, body wall 2.45%.

Among the four common genera, body wall holothurin content was highest in *Stichopus,* the three other genera with equivalent yield or in body wall holothrin content *Stichopus* > *Actinopyga* = *Bohadschia* = *Holothuria*. Gut holothurin content did not vary significantly among the genera although by Duncan's test it was shown to be higher in *Bohadschia.* Betwen *Holothuria* and *Bohadschia* with members possessing Cuvierian tubules, Cuvierian tubule holothurin content was higher in *Holothuria*

The different crude holothurins varied significantly in hemolytic potency, also, among the members of the families and genera and in the different parts of the body of the sea cucumbers. Hemolytic potency ranged from 571 to 666,667 HI/g with highest activity in the Cuvierian tubules. In decreasing order of potency, Cuvierian tubules > gut > body wall. The average HI/g for the different organs of the body were Cuvierian tubules 350,289, gut 114,705, body wall 65,465.

Table 1. Crude holothurin content in the body walls of sea cucumbers of different species with corresponding hemolytic potency.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED BODY WALLS, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
Family Holothuriidae							
Actinopyga echinites	1-26-84	LUSF1	16	31.2	0.0541	0.17	133,333
	1-26-84	LUSF1	17	37.2	0.3281	0.88	56,084
	1-26-84	LUSF1	18	73.7	0.1318	0.18	28,788
	1-26-84	LUSF1	19	74.7	0.2322	0.31	50,529
	2-21-84	QLpb	32	25.8	0.1150	0.44	72,438
	2-28-84	BCbbS	42	58.1	0.6410	1.10	85,714
	3-20-84	LUSF1	9	100.5	2,4791	2.47	134,898
	12-28-84	LUSF1	125	62.1	1,5057	2.43	115,873
	12-28-84	LUSF1	127	37.1	0.4795	1.29	56,168
	3-08-85	BCbbs	110	32.5	0.0885	0.27	–
	3-08-85	BCbbb	100	21.7	0.3925	1.81	126,495
Actinopyga mauritiana	3-09-85	BCbbb	131	32.7	0.6391	1.95	302,020
Actinopyga miliaris	2-21-84	QLpb	33	20.8	0.0830	0.40	33,333
	5-01-84	BCbbb	52	29.4	0.3730	1.27	50,159
	3-09-85	BCbbs	133	34.9	0.8540	2.45	118,518
Actinopyga sp.	2-01-84	BCbbb	22	45.7	1.1595	2.54	28,191
Bohadschia argus	2-28-84	BCbbb	39	112.9	4,1306	3.66	63,810
Bohadschia graeffei	12-22-83	LUSF1	3	38.8	0.4227	1.09	181,714
Bohadschia marmorata	2-01-84	Bcbbb	23	37.7	1.9255	5.11	11,259

Table 1. Continued.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED BODY WALLS, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
	2-28-84	BCbba	43	63.8	1.4432	2.26	40,000
	2-08-85	BCbbs	107	97.5	0.4257	0.44	124,444
	3-08-85	BCbbb	124	116.3	3.1558	2.71	–
Bohadschia vitiensis	12-20-83	LUSF1	1	77.5	0.4584	0.59	65,057
	2-28-84	PCbbb	38	71.0	0.2545	0.36	43,492
	5-13-84	PBsi	59	22.3	0.8518	3.82	–
	3-08-85	BCbbs	105	34.9	1.1606	3.33	17,801
	3-08-85	BCbbb	126	70.9	0.5740	0.81	76,623
Holothuria atra	12-18-83	LNK	20	81.8	0.6171	0.75	211,640
	12-18-83	LNK	116	35.4	1.8300	5.17	82,478
	2-28-84	BCbba	40	24.4	0.3430	1.41	36,565
	4-01-84	IGnv	57	225.4	1.9953	0.89	14,626
	4-01-84	IGnv	114	23.9	0.7132	2.98	63,491
	4-28-84	CNM	48	79.3	1.6993	2.14	151,111
	3-08-85	BCbbb	109	64.4	2.5697	3.99	41,481
	3-08-85	BCbbb	128	47.7	0.4043	0.85	82,222
	3-08-85	BCbbb	140	62.7	2.1853	3.49	–
	3-09-85	BCbbs	139	59.1	0.1946	0.33	122,843
Holothuria coluber	5-13-84	PBsi	55	53.6	1.0562	1.97	7,000
Holothuria fuscocinerea	2-21-84	QLpb	31	21.8	0.1110	0.51	24,813

Table 1. Continued.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED BODY WALLS, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
	5-01-84	BCbbb	53	20.3	0.7640	3.76	46,603
	5-13-84	PBsi	58	43.3	1.7942	4.14	29,919
Holothuria hilla	3-09-85	BCbbs	132	1.6	0.0445	2.78	50,217
Holothuria impatiens	7-15-83	LUSFp	7	109.7	0.4998	0.46	9,439
Holothuria klunzingeri	5-01-84	BCbbb	49	19.7	0.2388	1.21	20,571
Holothuria nobilis	5-13-84	PBsi	54	22.1	1.5202	6.88	8,169
Holothuria pervicar	12-22-83	LUSFp	2	67.3	0.8912	1.32	32,064
Holothuria pulla	12-18-83	LNK	21	56.2	0.3639	0.65	190,476
	1-26-84	LUSF1	11	27.8	1.4125	5.23	
	3-30-84	IGnv	56	103.0	1.3864	1.35	30,769
	3-30-84	IGnv	112	4.7	0.1203	2.56	35,353
	5-01-84	BCbbb	51	82.3	0.8427	1.02	24,762
	3-08-85	BCbbb	108	33.3	1.1259	3.38	120,000
	3-08-85	BCbbb	104	191.7	6.8776	3.59	223,703
	3-08-85	BCbbb	129	24.6	1.5501	6.30	44,815
Holothuria rigida	2-28-84	BCbba	45	18.4	0.3277	1.78	24,550
Holothuria sanguinolenta	1-10-82	AT	13	60.0	1.4525	2.42	78,307
Holothuria scabra	2-01-84	BCbbb	26	203.8	1.6674	0.82	76,191
	2-28-84	BCbba	41	60.2	0.7323	1.22	60,318
	12-01-84	BCbbs	106	199.1	3.9148	1.97	4,027

Table 1. Continued.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED BODY WALLS, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
	3-08-85	BCbbs	130	101.9	3.5392	3.47	123,565
	3-08-85	BCbbs	136	196.3	5.5016	2.80	–
Holothuria tigris	1-10-82	AT	14	120.8	2.2223	1.84	5,926
Family Stichopodidae							
Stichopus chloronotus	5-01-84	Bcbb	50	24.7	0.8104	3.28	12,445
Stichopus naso	12-26-83	LUSF1	5	46.8	1.1418	2.44	6,753
Stichopus sp.	4-23-84	CNM	46	40.6	1.3482	3.32	14,730
Stichopus variegatus	2-01-84	BCbbb	25	7.7	0.1400	1.82	–
	2-20-84	QLpb	34	39.9	2.3800	5.97	571
	2-21-84	QLpb	35	27.4	0.9853	3.60	19,619
	2-21-84	QLpb	36	20.7	0.5078	2.45	571
	2-21-84	QLpb	37	17.6	0.8504	4.83	387,879
	2-28-84	BCbbb	44	62.9	1.4967	2.38	1,852
	4-23-84	CNM	47	41.3	3.0330	7.34	7,757
	3-09-85	BCbbb	138	19.5	1.4323	7.35	37,836
Stichopus variegatus	12-26-84	LUSF1	4	49.2	0.8418	1.71	8,824
var hermanii	12-26-84	LUSF1	6	101.0	0.7217	0.71	34,285
Family Synaptidae							
Opheodesoma grisea	3-08-85	BCbbb	64	36.3	2.3528	6.48	6,624

Table 1. Continued.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED BODY WALLS, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
Pendekaplectana nigra	3-08-85	BCbb	60	21.7	1.4914	6.87	5,238
Synapta maculata	3-08-85	BCbb	63	101.8	3.2096	3.15	1,564
Family Chiridotidae							
Polycheir rufescens	7-15-85	LUSFp	8	153.6	0.3319	0.22	39,682

[a]AT Tiwi, Albay
BCbba Balongbato (Alvares Farms) Calatagan, Batangas
BCbbb Balongbato (Burot Point), Calatagan, Batangas
BCbbs Balongbato (Sandbar), Calatagan, Batangas
CNM Mercedes, Camarines Norte
IGnw Nueva Valencia, Guimaras, Iloilo
LNK Kauswagan, Lanao del Norte
LUSFl Lingsat, San Fernando, La Union
LUSFp Poro, San Fernando, La Union
PBsi Silaqui Island, Bolinao, Pangasinan
QLpb Padre Burgos, Lucena, Quezon
[b]DW Dry weight basis
-Extract unavailable for assay

Table 1. Crude holothurin content in the gut of sea cucumbers of different species with corresponding hemolytic potency.

SPECIES	DATE AND PLACE[a] OF COLLECTION		EXTRACT NO.	DRIED GUT, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
Family Holothuriidae							
Actinopya echinites	1-26-84	LUSF1	77(18)	4.4	0.2906	6.60	13,395
	1-26-84	BCbbs	80(42)	6.8	0.4216	6.20	93,333
Actinopya miliaris	2-21-84	QLpb	152(33)	7.3*	0.2003	2.74	143,834
	5-01-84	BCbbb	96(52)	0.1	0.0155	15.50	111,111
Actinopyga sp.	2-01-84	BCbbb	99(22)	3.1	0.1275	4.11	101,058
Bohadschia argus	2-28-84	BCbbb	65(39)	3.8	0.3420	9.00	-
	2-28-84	BCbbb	75(39)	5.0	0.211	44.23	324,786
Bohadschia graeffei	12-22-83	LUSFl	72(3)	6.1	0.5919	9.70	17,801
Bohadschia marmorata	2-01-84	BCbbb	97(23)	2.4	0.0638	2.66	77,576
	2-28-84	BCbba	81(43)	21.8	1.7080	7.83	342,857
	3-08-85	BCbbb	118(124)	11.7	0.6072	5.19	414,175
	3-08-85	BCbbs	141(107)	17.4	1.9027	10.94	444,444
Bohadschia vitiensis	12-20-83	LUSF1	85(1)	1.3	0.1763	13.56	61,838
	2-28-84	BCbbb	82(38)	10.6	1.3248	12.50	505,291
	3-08-85	BCbbb	122(126)	5.1	0.7056	13.84	57,143
Holothuria atra	12-18-83	LNK	92(20)	12.4	0.4853	3.91	77,576
	2-28-84	BCbba	73(40)	4.7	0.1831	3.90	20,571
	3-08-85	BCbbb	120(109)	2.6	0.0743	2.86	123,810

Table 2. Continued.

SPECIES	DATE AND PLACE OF COLLECTION[a]		EXTRACT NO.	DRIED GUT, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT, DW BASIS[b]	HI/g
Holothuria pervicax	12-22-85	LUSFp	86(2)	8.6	0.2634	3.06	37,714
Holothuria pulla	12-18-83	LNK	90(21)	16.1	0.5384	3.34	87,777
	1-26-84	LUSF1	79(11)	4.8	0.3519	7.33	8,667
	5-01-84	BCbbb	70(51)	7.5	0.3573	4.76	26,032
Holothuria rigida	2-28-84	BCbba	84(45)	0.4	0.0203	5.51	309,524
Holothuria scabra	2-01-84	BCbbb	101(26)	1.7	0.0741	4.36	8,667
	2-01-84	BCbbb	151(26)	12.1	0.0567	4.69	53,333
	2-28-84	BCbba	74(41)	2.8	0.0497	5.35	9,259
	12-01-84	BCbba	121(106)	2.2	0.0636	2.89	91,429
Family Stichopodidae							
Stichopus naso	12-26-83	LUSF1	87(5)	0.8	0.01808	22.60	3,302
Stichopus sp.	4-23-84	CNM	94(46)	1.1	0.1186	10.78	5,714
Stichopus variegatus	2-01-84	BCbbb	95(25)	1.0	0.0181	1.81	13,333
	2-21-84	QLpb	98(36)	1.1	0.0460	4.18	9,196
	2-28-84	BCbbb	83(44)	1.5	0.0534	3.56	29,815
Stichopus variegatus var heranni	12-26-84	LUSF1	88(4)	3.9	0.1765	4.53	44,502
	12-26-84	LUSF1	89(6)	2.5	0.0931	3.72	

[a]See Table 1
[b]Dry weight basis
[c]Gonads- Extract unavailable for assay

Table 3. Crude holothurin content in the Cuvierian tubules of sea cucumbers of different species with corresponding hemolytic potency.

SPECIES	DATE AND PLACE OF COLLECTION		EXTRACT NO.	DRIED CUVIERIAN TUBULES, (g)	CRUDE HOLOTHURIN, (g)	% CRUDE HOLOTHURIN CONTENT DW BASIS[b]	HI/g
Family Holothuriidae							
Bohadschia argus	2-28-84	BCbbb	143(39)	7.4	0.0370	0.50	666,667
Bohadschia graeffei	12-22-83	LUSF1	145(3)	9.4	0.0162	0.17	523,809
Bohadschia marmorata	2-01-84	BCbbb	155(23)	9.3	0.2326	2.50	304,762
	2-28-84	BCbba	146(43)	37.4	0.9200	2.46	666,667
Bohadschia vitiensis	12-20-83	LUSF1	147(1)	17.0	0.0756	0.45	592,593
	2-28-84	BCbbb	144(38)	21.3	0.3155	1.48	263,492
	5-13-84	PBsi	154(59)	6.3	0.3727	5.92	-
	3-08-85	BCbbb	153(126	12.9	0.5050	3.92	457,142
	3-08-85	BCbbs	161(105)	8.2	0.4739	5.78	-
Holothuria fuscocinerea	5-01-84	BCbbb	162(53)	9.9	0.8567	8.67	101,587
	2-21-84	QLpb	150(31)	6.9	0.7386	10.70	16,212
Holothuria pervicax	12-22-83	LUSFp	157(2)	11.2	0.1506	1.34	91,967
Holothuria pulla	12-18-83	LNK	165(21)	106.4	6.0971	5.73	-
	1-26-84	LUSF1	156(11)	6.9	1.0387	15.05	-
	5-01-84	BCbbb	142(51)	12.2	0.1590	1.30	114,286
	3-08-85	BCbbb	164(108)	6.6	0.1639	2.48	-

[a]See Table 1.
[b]Dry weight basis.
-Extract unavailable for assay.

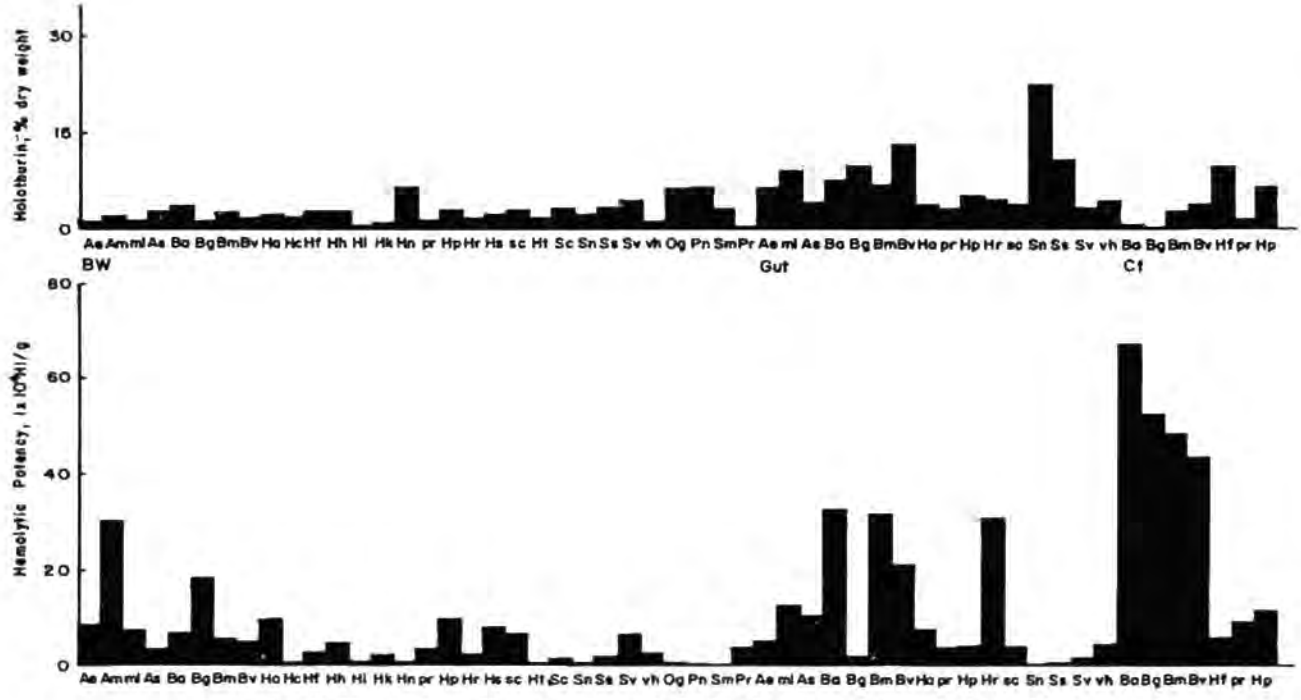

Fig. 1. The ethanolic extracts of Philippine sea cucumbers with corresponding hemolytic potency.

Body wall holothurin hemolytic activity gave this order of potency: Holothuriidae > Stichopodidae > Synaptidae = Chiridotidae with members of the same family and genera exhibiting equal potencies or among Family Holothuriidae, *Actinopyga* = *Bohadschia* = *Holothuria.* However, gut holothurin gave this order of potency: *Bohadschia* > *Actinopyga* > *Holothuria* > *Stichopus* while Cuvierian tubule holothurin gave *Bohadschia* > *Holothuria.*

Analysis on the data of the hemolytic assay showed that time and locality of collection were insignificant factors for variation. The Duncan's test showed, however, slightly higher activities during January to March of each year from 1983-1985, higher than in midyear or July.

The present comparative study agrees with an initial observation of the potency of *Bohadschia* (Pocsidio, 1983), likewise, with the report of the Russians based on its antifungal activity (Baranova *et al.*, 1973; Shcheglov *et al.*, 1979; Kuznetsova *et al.*, 1982). It found a lower potency of *Stichopus* which probably could be explained by a low stichoposide content which was revealed recently in thin layer chromatography plates of the extracts.

The results of the hemolytic assay identify the potent sources of holothurin from Philippine holothurians. The finding that the more commonly and more abundantly found sea cucumbers are sufficient sources makes further studies on holothurin relevant. Hitherto, the species which had been ignored because of lack of palatability, for example, *Holothuria pulla,* may gain economic importance as source of holothurin. The absence of significant differences in the amounts of holothurin according to seasonal changes and the distribution of the animals in the archipelago also contribute to the feasibility of research on the ``material medica".

The claim of Chinese traditional medicine of the effectiveness of the sea cucumber to :"thin" out blood and reduce hypertension seems convincing. When ingested, holothurin is expected to be hydrolyzed. Some complete holothurin could be absorbed from the gut due to their lipoidal solubility.

Possibly, the greater the polarity of the glycosides, the less effective is the capacity for cholesterol abstraction. The lesser is the polarity, the more effective is the hydrophobic bonding. Thus, the hemolytic potency of the sea cucumbers of the genera *Bohadschia, Holothuria,* and *Actinopyga* of the family Holothuriidae may be due to such holothurins as bivittoside A, Holothurin B, and echinoside B. Among these three sea cucumber glycosides, the absence of a sulfate group in bivittoside A would make a difference in the holothurin activity.

SUMMARY

I. Results of statistical analysis on the data on 79 samples of the body wall, 34 samples of gut, and 16 samples of Cuvierian tubules:

1. Crude holothurin content of the different sea cucumbers in the different parts of their body vary significantly in amount. Range in amounts on dry weight basis - 0.17% to 22.60%. Gut > Cuvierian tubules > Body wall. Average % crude holothurin, dry weight basis - Gut 6.64%, Cuvierian tubules 4.285%, Body wall 2.48%
2. Among four genera *Actinopyga, Bohadschia, Holothuria,* and *Stichopus*
   2.1 Body wall holothurian content - *Stichopus* > *Actinopyga* = *Bohadschia* = *Holothuria*
   2.2 Gut holothurin content - no significant differences although by Duncan's test higher in *Bohadschia*
3. Between two genera *Holothuria* and *Bohadschia* Cuvierian tubule holothurin content - higher in Holothuria

II. Results of statistical analysis on data on 72 samples of the body wall, 32 samples of gut, and 11 samples of Cuvierian tubules.

1. Hemolytic activity of crude holothruin of different sea cucumbers in the different parts of their body vary significantly. Range in potency, HI/g-571 to 666,667 HI/g - Cuvierian tubules 350,289, Gut 114,705 Body wall 65,465
2. Cuvierian tubule holothurin hemolytic activity - *Bohadschia* > *Holothuria*
3. Gut holothurin hemolytic activity -- *Bohadschia* > *Actinopyga* > *Holothuria* > *Stichopus*
4. Body wall holothurin hemolytic activity -- Holothuriidae > Stichopodidae > Synaptidae = Chiridotidae
5. No significant differences in hemolytic potency of body wall, gut, or Cuvierian tubule holothurin among species belonging to the same genus or family
6. Time of collection is an insignificant factor for variation although by Duncan's test, higher activities during January to March of each year from 1983 to 1985, higher than in mid year or July
7. Locality of collection is an insignificant factor for variation

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