Zoological Studies **37**(2): 102-110 (1998)

Zoological Studies

Conversion of Cholesterol to Sex Steroid-like Substances by Tissues of *Mictyris brevidactylus* in Vitro

Jin-Taur Shih[1,*] and Ching-Fong Liao[2]

[1]*Department of Biology, National Taiwan Normal University, Taipei, Taiwan 117, R.O.C.*
[2]*Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, R.O.C.*
Tel: 886-2-29333149 ext. 339. Fax: 886-2-29312904.

(Accepted January 20, 1998)

Jin-Taur Shih and Ching-Fong Liao (1998) Conversion of cholesterol to sex steroid-like substances by tissues of *Mictyris brevidactylus* in vitro. *Zoological Studies* **37**(2): 102-110. This study aimed to determine whether the ovary or hepatopancreas of the soldier crab *Mictyris brevidactylu*s can synthesize sex steroid-like substances. Tritium-labeled cholesterol was used as a precursor for steroid synthesis and was added to the culture medium in which tissues were incubated in vitro. Steroid extracts prepared from ovaries, hepatopancreases, and their incubation media were analyzed by HPLC. We had the following results. 1) Ovary and hepatopancreas took up tritium-labeled cholesterol at different rates and these rates leveled off during incubation. 2) By HPLC analysis, we detected tritium-labeled radioactivities in fractions which might represent newly synthesized steroid-like substances (17-α-hydroxypregnenolone, progesterone, and pregnenolone). 3) Little or no tritium-labeled steroid-like substance was detected in tissues taken from crabs during prereproductive and reproductive seasons (egg-carrying).

Key words: Crab, Ovary, Hepatopancreas, Sex steroids.

Whether crustaceans produce endogenous steroid hormones remains untested. However, sex steroid-like substances and enzymes involved in steroid metabolism have been detected in tissues of crab and shrimp (Lisk 1961, Kanazawa and Teshima 1971, Teshima and Kanazawa 1971, Sandor 1981, Couch and Hagino 1983, Skinner 1985, Couch et al. 1987, Fingerman 1987, Sasser and Singhas 1988 1992, Shih 1992 1993 1997, Shih and Wang 1993). Physiological studies have shown that progesterone and 17-α-hydroxyprogesterone increased yolk protein synthesis in ovaries of penaeid shrimp (Kulkarni et al. 1979, Quackenbush and Keeley 1987 1988, Quackenbush 1992). The maturation of penaeid shrimp ovary was enhanced by progesterone and 17-α-progesterone (Sarojini et al. 1985 1986, Yano 1987). Quackenbush (1994) stated that steroid hormones produced in lobster ovary may regulate yolk production.

Shih (1992 1993), and Shih and Wang (1993) reported that in the hemolymph progesterone-like substance levels of *Uca arcuata* and *Mictyris brevidactylus* reached the peak phase 2 to 3 mo before their egg-carrying periods (April-May for *U. arcuata*, January-April for *M. brevidactylus*) started. At the same time, the gonadosomatic index (GSI) also reached its peak phase. This suggests that the progesterone-like substance is an endogenous product of the crab. Also this hormone may have an effect on crustacean reproduction, for example, in ovarian maturation as suggested by Quackenbush (1989a,b 1992).

Recently, Shih (1997) detected progesterone-like and estradiol-like substances in steroid extracts of ovary and hepatopancreas of *M. brevidactylus*. However, there is still a lack of evidence showing that sex steroids are synthesized in this crab. In this report, tritium-labeled cholesterol was used as a precursor to test if crab tissues could synthesize sex steroids in vitro. After incubation, steroid extracts of ovaries and hepatopancreases contained

*To whom correspondence and reprint requests should be addressed.

tritium labeled substances. HPLC analysis showed that labeled substances may represent newly synthesized steroids. Steroid extracts of tissues taken from nonreproductive season (August) specimens were labeled with more radioactivities than those of prereproductive (November and December) and reproductive seasons (January) specimens.

MATERIALS AND METHODS

Chemicals

Organic solvents used were LC grade from Alps Chemical Co. (Taipei, Taiwan, R.O.C.). The authentic steroids were from Sigma Co. (St. Louis, MO, USA).

Sample collection

Adult (carapace width > 0.60 cm) female soldier crab, *M. brevidactylus* were collected in the Tanshui mangrove swamp of northern Taiwan in nonreproductive (August), prereproductive (November and December), and reproductive (January) seasons (Shih et al. 1991, Shih 1993). Zero-year-old crabs (CW < 0.50 cm) were collected in the nonreproductive season (August).

In vitro incubation of tissues

Crabs were rinsed and anesthetized in a refrigerator (4 °C). Ovaries and hepatopancreases were dissected out and weighed. About 18-36 mg of tissue was usually placed in an Eppendorf vial (2.0 ml) filled with saline (Shih et al. 1989). For labeling experiments, tritium-labeled cholesterol [1α, 2α(n)-tritium, 48.0 Ci/mmol, Amersham International Place, UK] was added to the incubation medium (0.5-0.7 μCi/[ml of saline]). For study of uptake kinetics, a lesser radioactivity (0.06 μCi/[ml of saline]) was used. After incubating at 22.0 °C for 20-24 h with shaking (80 rpm/min), tissues were rinsed twice each in 20 ml of cold saline and placed in absolute ethanol.

Extraction of steroids

Extraction of steroids from ovary and hepatopancreas tissues was carried out by using a modified procedure described previously (Shih 1992, Shih and Wang 1993). Ovaries or hepatopancreases were homogenized twice in cold absolute ethanol in a mortar. The pooled homogenate was filtered. The filtrate was extracted twice with 10 volumes of methanol and chloroform (1:2, v/v). The organic phase was air-dried. This extract was dissolved in 0.3 M $CaCl_2$ in 70% methanol. This mixture was kept in a refrigerator (4 °C) overnight. The precipitate was removed by filtering. The above procedure was repeated once. The steroids in the aqueous phase were extracted by dichloromethane. To 40 ml dichloromethane extract, 8 ml water, 8 ml 0.1 N NaOH, and 8 ml 0.1 N acetic acid were added and mixed in a separatory funnel. After removing the aqueous phase, the extract in the dichloromethane was evaporated to dryness. The resulting extract was designated as the steroid extract and used for this study. Because the incubation medium (saline) contained water, it was necessary to separate the aqueous phase by adding diethyl ether to the medium. The ether phase containing steroids was collected; after the ether was evaporated, the extract was dissolved in absolute ethanol and subjected to the same procedure as for the ovaries.

High-performance liquid chromatography

The HPLC system used for this study was

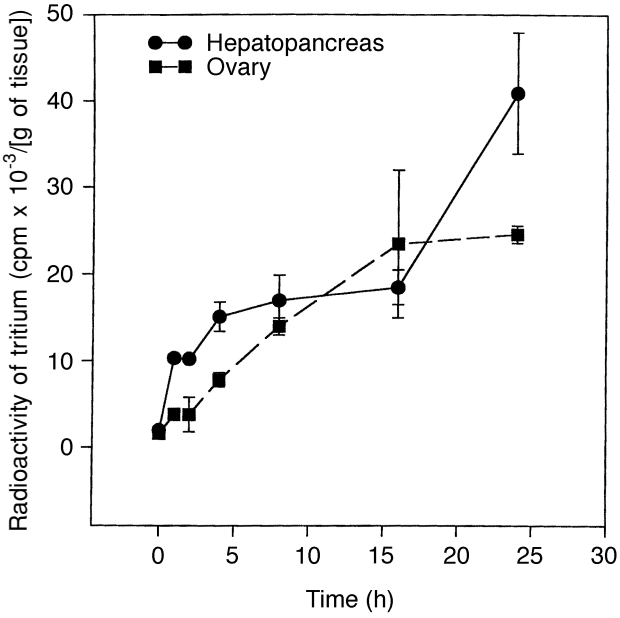

Fig. 1. Uptake kinetics of tritium-labeled cholesterol by tissues of *Mictyris brevidactylus* in vitro. Ovaries or hepatopancreases (August 1996) were incubated in 2.0 ml saline with tritium-labeled cholesterol (0.06 μCi/[ml of saline]) at 20 °C for 24 h. At each time point, tissues were rinsed with 20.0 ml saline twice and fixed in absolute ethanol. Then tissue was homogenized, air-dried, and prepared for scintillation counting. The bar of each point represents the mean and standard errors (n = 2).

composed of a Knauer HPLC pump (Type 364, Germany), a sample injector, a Spectral photometer (N_r/No_o. 731 879), a Chromatocorder 11 (SIC, Japan) and a column of LiChroCART 250-4, RP-C18 (4.0 mm × 244 mm, Merck, Germany). The elution was carried out by the procedure of Shih et al. (1990). Methanol at 80% was first used to separate steroid hormones, followed by 100% methanol to elute the cholesterol.

If not otherwise stated, UV-absorbing steroids were detected in all chromatograms at 275 nm; attenuation was set at 32 a.u./cm. A sample of 20 μl was injected for each run. Steroid extracts were dissolved in 0.6-1.0 ml methanol, filtered through a Millipore membrane (0.45 μm) and prepared for HPLC. Authentic steroid standards were progesterone (pregn-4-one-3, 20-dione), pregnenolone (5-pregnen-3β-ol-20-one), 17-α-hydroxypregnenolone (5-pregnene-3β,17α-diol-20-one), and cholesterol. Eluates which had retention times corresponding to standards were collected at the OD peak region. Fractions without OD peaks were also collected at various time intervals. All eluates were extracted with ether and then air-dried. The extract of each eluate was first dissolved in 0.5 ml 95% ethanol and then mixed with 6.0 ml Omni-Szintisol (Merck, Darmstadt, Germany) in preparation for liquid scintillation counting.

To check the efficiency of the extraction procedure and the possibility of cholesterol metabolism, tritium-labeled cholesterol (0.5 μCi) was mixed with 10.0 ml saline and stored in an incubator (22.0 °C) for 24 h. This mixture was then extracted for steroids. The efficiency of extraction was 79.6%. When this tritium-labeled cholesterol extract was run through HPLC, 76.0%-79.0% of the applied radioactivity was recovered at the retention time of authentic cholesterol, and 21.0%-24.0% of the radioactivity came out a few minutes before the cholesterol did.

RESULTS

Different cholesterol uptake rates by the ovary and hepatopancreas

Fig. 1 shows the uptake over time of tritium-

Table 1. Radioactivity detected in tissue, medium, and their steroid extracts after *Mictyris brevidactylus* fresh tissues were incubated in the presence of tritium-labeled cholesterol[a] in vitro

Date of sampling and experiment	Radioactivity (% of total input) detected in		Radioactivity (% of total input) detected in steroid extract of	
	Tissue	Medium	Tissue	Medium
1995				
November				
Ovary	5.55 ± 0.64[b]	65.76 ± 5.34	3.66 ± 0.55	60.32 ± 6.44
Hepat.[c]	1.77 ± 0.45	71.66 ± 7.22	2.44 ± 0.51	51.11 ± 7.12
December				
Ovary	1.20	61.31	0.14	36.10
Hepat.	1.71	66.61	0.52	32.76
1996				
January				
Ovary	4.31	72.05	2.00	49.32
Hepat.	2.22	81.52	1.20	33.11
August (I)[d]				
Ovary	–[e]	–	1.27	33.64
Hepat.	–	–	1.31	36.60
August (II)[f]				
Hepat.	–	–	2.91	56.96

[a] Radioactivity of cholesterol applied to medium (2.0-4.0 ml) was 0.5-0.7 μCi/[ml of saline].
[b] Results are from 2 separate experiments and expressed as mean and standard errors.
[c] The abbreviation Hepat. represents hepatopancreas of crab.
[d] Tissues of adult crab (CW > 0.60 cm).
[e] Not determined.
[f] Tissues of 0-yr-old crab (CW < 0.50 cm) which had premature ovaries.

labeled cholesterol by the ovary and hepatopancreas of *M. brevidactylus*. The uptake of tritium-labeled cholesterol by the ovary reached a maximal level before 15 h, and maintained a plateau for another 10 h. The uptake kinetics of tritium-labeled cholesterol by the hepatopancreas were more complex, reaching a maximal level before 5 h and maintaining a plateau for another 10 h; then the uptake increased to a higher level thereafter. After incubation, the ovary took up 1.45% (2.5×10^5 CPM/[g of ovary]) of the total input radioactivity while the hepatopancreas took up 2.50% (4.1×10^5 CPM /[g of hepatopancreas]).

Table 1 shows the amount of radioactivities in the tissues and incubation media. At the end of incubation, about 1.20%-5.55% of the total input radioactivity was found in the tissues. After extraction for steroids, the tissue and medium extracts had 0.14%-3.66% and 32.0%-60.0% of the total input radioactivity, respectively.

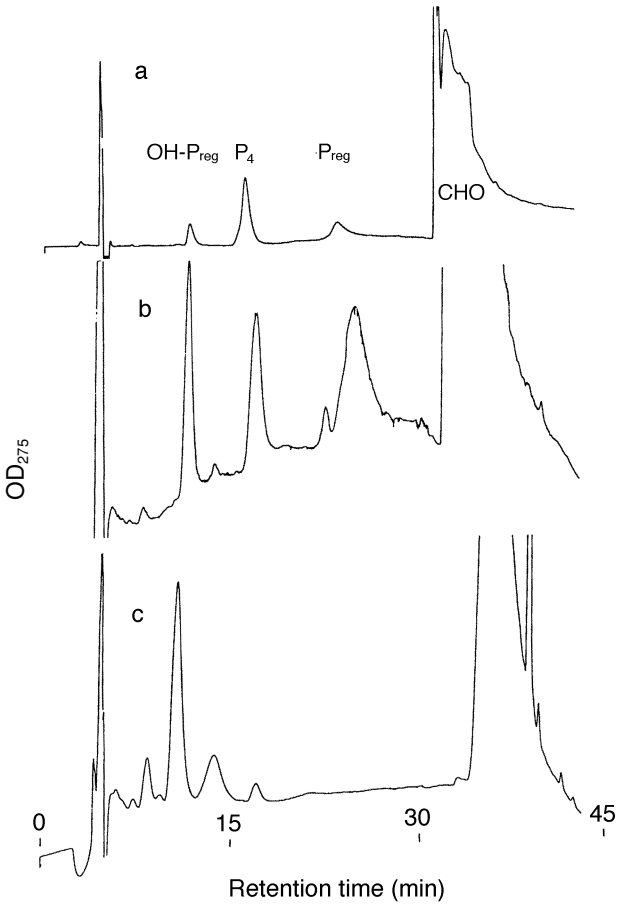

Fig. 2. HPLC chromatograms for steroid standards and samples. (a) Authentic steroid standards were 17-α-OH-pregnenolone (OH-Preg), progesterone (P_4), pregnenolone (Preg), and cholesterol (CHO, 1000 ng each, except P_4 at 150 ng). (b) Tritium-labeled steroid extract of *Mictyris brevidactylus* ovary (August 1996) in the presence of 4 authentic steroids as analyzed by HPLC. (c) Tritium-labeled steroid extract of (b) in the absence of steroid standards as analyzed by HPLC.

HPLC analysis of steroid extracts of tissue and incubated medium

Fig. 2a is a typical chromatogram of authentic steroids: 17-α-hydroxypregnenolone (OH-P_{reg}), progesterone (P_4), pregnenolone (P_{reg}), and cholesterol (CHO, 1000 ng each, except P_4 at 150 ng). Retention times of these standards were 11.5 min, 18.0 min, 27.1 min, and 33.8-37.0 min, respectively. After that the tritium-labeled ovary steroid extract (sample prepared in August [I] 1996, see Table 1) passed the HPLC with 4 authentic steroids; its chromatogram is shown in Fig. 2b. The authentic steroids still had correlated retention times with those in Fig. 2a (11.7 min, 17.8 min, 27.3 min, and 35.0-38.0 min). The ovary extract run through HPLC without standards is shown in Fig. 2c. Three OD peaks with retention times of 11.0 min, 17.6 min, and 33.0-38.0 min may represent OH-P_{reg}, P_4, and CHO, respectively.

Radioactivity detected in chromatographed steroid fractions

Labeled steroid-like substances of the ovary steroid extract

As shown in Fig. 2b, fractions of the whole profile of the chromatogram were collected. The OD and radioactivity of each fraction were determined and recorded. Results are shown in Table 2. Based on the retention times, 3.66%, 3.74%, and 7.42% of the chromatographed radioactivity were found in fractions which may represent newly synthesized OH-P_{reg}, P_4, and P_{reg}, respectively. Some radioactivities were also found in the fractions which came out between the authentic steroid standards. Lower radioactivity was detected when the medium steroid extracts were run through HPLC (0.75%-0.90% of the chromatographed radioactivity). The fraction which contained cholesterol had relatively high radioactivity (48.89%-85.70%).

Labeled steroid-like substances of the hepatopancreas steroid extract

Tritium-labeled hepatopancreas steroid extract was run through HPLC, and the OD and radioactivity of each fraction are shown in Table 3. There were 3.45%, 5.45%, and 4.70% of the chromatographed radioactivity detected which may repre-

sent labeled OH-P_{reg}, P_4, and P_{reg}, respectively. Again, lower radioactivities (0.29%-1.82%) were found in steroid fractions when the medium steroid extracts were analyzed.

Labeled steroid-like substances of tissues and media during the study period

Table 4 presents results of the chromatographed tissue and medium steroid extracts which were labeled with tritium. Though radioactivities were detected in steroid fractions of ovaries and their media of November and December 1995 and January 1996, they were at low levels (< 0.92% of the chromatographed radioactivity, except 1.62% of ovary medium of December 1995). No radioactivity was detected in the hepatopancreas steroid extracts of December 1995 and January 1996. Table 4 also presents the results of experiments conducted in August 1996 with the hepatopancreases of 0-yr-old crabs which had only premature ovaries (barely detected tissue). About 3.79%, 5.28%, and 3.31% of the chromatographed radioactivity were found in fractions representing OH-P_{reg}, P_4, and P_{reg}, respectively.

DISCUSSION

There are 3 main findings of this study. 1) Ovary and hepatopancreas took up tritium-labeled cholesterol in vitro. 2) By HPLC analysis, tritium-labeled radioactivities were detected in fractions which possibly represent the newly synthesized steroid-like substances 17-α-hydroxypregnenolone, progesterone, and pregnenolone. 3) Little or no tritium-labeled steroid-like substance was detected in tissues taken from specimens of late prereproductive (November and December) and reproductive (January) seasons.

According to Van Den Oord (1964), and Zandae (1967), crustaceans do not synthesize cholesterol de novo; tritium-labeled cholesterol was therefore used in this study as a precursor for steroid synthesis by crab tissues in vitro. After incubation in vitro for 24 h, tissues took up 1.20% to 5.55% of the radioactivity applied to the incubation medium. Watson and Spaziani (1985) used ^{14}C-labeled cholesterol (1.0 μCi/[ml of saline]) to study the synthesis of ecdysone by crab tissues. Similarly, these tissues took up about 1.0% to 5.0% of

Table 2. Radioactivity of eluate fractions from HPLC of *Mictyris brevidactylus* ovary steroid extract[a]

Fraction no.	OD_{275}	Retention time (min)	Radioactivity (CPM) of eluate of tissue (T) and medium (M) extract	Percent of total chromatographed radioactivity	Steroid standard emerging at this retention time
1[b]	0.003	5 – 10	468 (T)[c] 374 (M)	4.76 1.31	
2	0.008	10 – 12	360 (T) 254 (M)	3.66 0.90	OH-Pregc[d] OH-Preg
3	0.003	12 – 17	189 (T) 270 (M)	1.92 0.95	
4	0.002	17 – 19	368 (T) 218 (M)	3.74 0.76	P_4 P_4
5	0.000	19 – 26	516 (T) 100 (M)	5.24 0.03	
6	0.000	26 – 28	730 (T) 214 (M)	7.42 0.75	Preg Preg
7	0.000	28 – 30	288 (T) 80 (M)	2.92 0.02	
8	> 0.040	30 – 35	2113 (T) 2560 (M)	21.47 8.99	Unk Unk
9	> 0.040	35 – 45	4810 (T) 24400 (M)	48.89 85.70	CHO CHO

[a] Ovaries of *M. brevidactylus* collected in August 1996 were incubated in saline with tritium-labeled cholesterol at 20 °C for 24 h. After incubation, tissue and its medium were extracted for steroids. Steroid extracts were then analyzed by HPLC.
[b] Fractions 1-7 came out in 80% methanol elution. Fraction 8-9 came out in 100% methanol elution.
[c] Abbreviations T and M represent tissue and medium, respectively.
[d] Abbreviations OH-Preg, P_4, Preg, Unk, and CHO represent 17-α-OH-pregnenolone, progesterone, pregnenolone, unknown substance, and cholesterol, respectively.

the total applied radioactivity. The uptake of labeled cholesterol by crab ovary leveled off at 24 h, while the Y-organ may have had a longer uptake time. Since the amounts of free cholesterol and the cholesteryl esters were not assayed in this study, the radioactive cholesterol taken up by *M. brevidactylus* tissues may contain free cholesterol. Different patterns of tritium-labeled cholesterol uptake rates may represent different metabolic mechanisms in the tissues (Fig. 1). In crab, circulating cholesterol may be bound to high-density lipoprotein (HDL), and cholesterol is taken up by endocytosis of the entire HDL-cholesterol complex (Kang and Spaziani 1995). Addition of crab serum may enhance the uptake process as shown by Watson and Spaziani (1985). In this study, unlabeled cholesterol (50 fold of the amount of the tritium-labeled cholesterol) was added into the culture medium as a chaser to reduce the incorporation of tritium-labeled cholesterol by the tissue. Results did not show any difference between control and test experiments analyzed by HPLC for the labeled steroids (data not shown).

In this study, the first 3 intermediates of the sex steroid synthesis pathway were analyzed by HPLC. When steroid extracts with or without steroid standards passed the HPLC, the OD peaks with retention times of 17-α-hydroxypregnenolone, progesterone, pregnenolone, and cholesterol were clearly separated on the chromatogram. Since fractions along the whole profile of the chromatogram were collected, the percentage of the radioactivity in each fraction was obtained. Results show that all 3 steroid-like substances were labeled with tritium, indicating the ovary and hepatopancreas possibly synthesize steroid-like substances.

The steroid extract of the culture medium also contained tritium-labeled steroid-like substances. This result indicates that the newly synthesized steroid-like substances were secreted into the medium. But the possible contamination of tissue to the culture medium could not be excluded.

Table 3. Radioactivity of eluate fractions from HPLC of *Mictyris brevidactylus* hepatopancreas steroid extract[a]

Fraction no.	OD_{275}	Retention time (min)	Radioactivity (CPM) of eluate of tissue (T) and medium (M) extract	Percent of total chromatographed radioactivity	Steroid standard emerging at this retention time
1[b]	0.002	5 – 10	1270 (T)[c]	5.02	
			120 (M)	1.10	
2	0.010	10 – 12	875 (T)	3.45	OH-Preg[d]
			32 (M)	0.29	OH-Preg
3	0.002	12 – 17	905 (T)	3.58	
			50 (M)	0.46	
4	0.008	17 – 19	1380 (T)	5.45	P_4
			74 (M)	0.62	P_4
5	0.001	19 – 26	1448 (T)	5.72	
			68 (M)	0.62	
6	0.006	26 – 28	1330 (T)	4.70	Preg
			198 (M)	1.82	Preg
7	0.000	28 – 30	1021 (T)	4.03	
			102 (M)	0.93	
8	> 0.040	30 – 35	7534 (T)	29.78	Unk
			2620 (M)	24.17	Unk
9	> 0.040	35 – 45	9534 (T)	37.68	CHO
			6404 (M)	58.90	CHO

[a] Hepatopancreases of *M. brevidactylus* collected in August 1996 were incubated in saline with tritium-labeled cholesterol at 20 °C for 24 h. After incubation, tissue and its medium were extracted for steroids. Steroid extracts were then analyzed by HPLC.

[b] Fractions 1-7 came out in 80% methanol elution. Fractions 8-9 came out in 100% methanol elution.

[c] Abbreviations T and M represent tissue and medium, respectively.

[d] Abbreviations OH-Preg, P_4, Preg, Unk, and CHO represent 17-α-OH-pregnenolone, progesterone, pregnenolone, unknown substance, and cholesterol, respectively.

Shih (1993) reported that a progesterone-like substance was detected in hemolymph of female *M. brevidactylus* at high levels 2-3 mo before egg-carrying. In a recent report, progesterone-like substance was detected in the hepatopancreas and ovary of *M. brevidactylus* (Shih 1997). In this study, tissues of August 1996 specimens converted radioactive cholesterol to sex steroid-like substances in vitro, indicating that these tissues may be the site of steroid-like substance synthesis. However, tissues taken from specimens of December 1996 and January 1997, which are times close to egg-carrying, had low or no activity to convert cholesterol to sex steroids.

Ovaries and hepatopancreases of the fiddler crab, *Uca vocans borealis* which has a shorter oogenesis time (about 2 wk) were tested for steroid synthesis in vitro in this laboratory. Preliminary results showed that these tissues synthesized tritium-labeled steroid-like substances at a relatively faster rate (data not shown).

In order to check if there was steroid hormone contamination between tissues, hepatopancreases of 0-yr-old crabs which had only immature ovaries were also tested. Results showed that these hepatopancreases synthesized even more tritium-labeled steroids in vitro. Therefore, it seems that both the ovary and hepatopancreas can independently synthesize steroid-like substances.

Steroid extracts of ovaries and hepatopancreases of *M. brevidactylus* were measured by radioimmunoassay for 17-α-OH-progesterone, aldosterone, and cortisol (Shih 1997). Results showed a positive immunoreaction to 17-α-OH-progesterone, but negative ones to aldosterone and cortisol. However, more quantitative data are needed to

Table 4. Radioactivity of fractions from HPLC of *Mictyris brevidactylus* ovary and hepatopancreas steroid extracts

Date of sampling and experiment	Tissue (T) or Medium (M)	Percentage of radioactivity in the fraction with retention time corresponding to standard steroid or cholesterol[a]				
		OH-Pre[b]	P_4	Pre	UNK	CHO
1995						
November	Ovary (T)	0.15	0.15	0.12	37.12	62.38
	Ovary (M)	0.16	0.38	0.34	42.17	56.18
	Hepat. (T)[c]	0.11	0.26	0.45	37.35	61.81
	Hepat. (M)	0.11	0.19	0.12	39.10	60.48
December	Ovary (T)	–[d]	–	–	–	–
	Ovary (M)	1.62	0.92	0.78	19.39	77.29
	Hepat. (T)	0.00	0.00	0.00	22.31	76.71
	Hepat. (M)	0.51	0.33	0.23	25.21	73.71
1996						
January	Ovary (T)	0.27	0.18	0.00	27.86	71.67
	Ovary (M)	0.06	0.07	0.07	21.87	77.91
	Hepat. (T)	0.00	0.00	0.00	38.90	61.10
	Hepat. (M)	0.06	0.15	0.05	19.62	80.10
August[e]	Hepat. (T)	3.79	5.28	3.31	11.28	78.38
	Hepat. (M)	1.24	0.90	0.87	3.74	87.45
	Saline with ^{3}H-cholesterol[f]	0.00	0.00	0.00	22.10	77.90

[a] HPLC was carried out as described in Materials and Methods. Radioactivities of ovary and hepatopancreas steroid extracts applied to HPLC were 2900 to 23 000 CPM. Radioactivities of tissue incubation media were 61 000 to 208 000 CPM. Fractions with retention times corresponding to authentic steroids were collected and prepared for radioactivity counting. Other fractions at 1-2 min intervals were also prepared for radioactivity counting (data not shown).

[b] Abbreviations OH-Pre, P_4, Pre, UNK, and CHO represent 17-α-OH-pregnenolone, progesterone, pregnenolone, unknown substance, and cholesterol, respectively.

[c] The abbreviation Hepat. represents hepatopancreas.

[d] Ovary steroid extract of December 1995 had low radioactivity and was not assayed by HPLC.

[e] Results are of tissues of 0-yr-old crabs which had premature ovaries.

[f] Saline with tritium-labeled cholesterol was stored at 22.0 °C for 24 h. Steroid extract was prepared from this saline and analyzed by HPLC.

support the possibility that this crab can synthesize sex steroids endogenously.

Acknowledgments: The authors wish to thank Dr. Shong Huang, Mr. R. C. Chang of the Department of Biology, National Taiwan Normal University, and Mrs. J. Shih for their suggestions and help. Thanks also go to the Department of Biology, NTNU, and National Science Council, R.O.C. (NSC-85-2311-B-003-009) for their financial support.

REFERENCES

Couch EF, N Hagino. 1983. Correlation of progesterone and estradiol production by the mandibular organ and other tissues with egg development in the American lobster. J. Cell Biol. **97:** 158A.

Couch EF, N Hagino, JW Lee. 1987. Changes in estradiol and progesterone immunoreactivity in tissues of the lobster, *Homarus americanus*, with developing and immature ovaries. Comp. Biochem. Physiol. **85A:** 765-770.

Fingerman M. 1987. The endocrine mechanisms of crustaceans. J. Crust. Biol. **7:** 1-27.

Kanazawa A, SI Teshima. 1971. *In vivo* conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonica*. Bull. Jpn. Soc. Sci. Fish. **37:** 891-898.

Kang BK, E Spaziani. 1995. Uptake of high-density lipoprotein by Y-organs of the crab *Cancer antennarius*: III. Evidence for adsorptive endocytosis and the absence of lysosomal processing. J. Exp. Zool. **273:** 425-433.

Kulkarni GK, R Nagabushanam, PK Joshi. 1979. Effect of progesterone on ovarian maturation in a penaeid prawn, *Parapenaeopsis dardwiakii* (Miers, 1978). Ind. J. Exp. Biol. **17:** 986-987.

Lisk RD. 1961. Estradiol-17-beta in the eggs of the American lobster, *Homarus americanus*. Can. J. Biochem. Physiol. **39:** 659-663.

Quackenbush LS. 1989a. Vitellogenesis in the shrimp, *Penaeus vannamei*. *In vitro* studies of the isolated hepatopancreas and ovary. Comp. Biochem. Physiol. **94B:** 253-261.

Quackenbush LS. 1989b. Yolk protein production in the marine shrimp, *Penaeus vannamei*. J. Crust. Biol. **9:** 509-516.

Quackenbush LS. 1992. Yolk synthesis in the marine shrimp, *Penaeus vannamei*. Comp. Bioch. Physiol. **103A:** 711-714.

Quackenbush LS. 1994. Lobster reproduction: a review. Crustaceana **67:** 82-94.

Quackenbush LS, LL Keeley. 1987. Vitellogenesis in the shrimp, *Penaeus vannamei*. Am. Zool. **26:** 810A.

Quackenbush LS, LL Keeley. 1988. Regulation of vitellogenesis in the fiddler crab, *Uca pugilator*. Biol. Bull. **175:** 321-331.

Sandor T. 1981. Steroids in invertebrates. *In* WH Clark, TS Adams, eds. Invertebrate reproduction. New York: Elsevier North Holland, pp. 81-96.

Sasser EW, CA Singhas. 1988. Presence of an estrogen-like compound in hemolymph samples of blue crab and *Penaeid* shrimp. Am. Zool. **28:** 118A.

Sasser EW, CA Singhas. 1992. Presence of an estriol-like steroid in the blue crab, *Callinectes sapidus*. Aquaculture **104:** 367-373.

Sarojini R, K Jayalakshmi, S Sambaahivarao. 1986. Effect of external steroids on ovarian development of freshwater prawn, *Macrobrachium limerrii*. J. Adv. Zool. **7:** 50-53.

Sarojini R, MS Mirajakar, R Nagabhushanam. 1985. Effect of steroids on oogenesis and spermatogenesis of the freshwater prawn, *Macrobrachium kistnensis*. Comp. Physiol. Ecol. **10:** 7-11.

Shih JT. 1992. Annual pattern of sex steroid-like substance levels in the hemolymph of female *Uca arcuata*. Bull. Inst. Zool., Acad. Sinica **31:** 47-56.

Shih JT. 1993. Annual patterns of gonadosomatic and hepatosomatic indexes and progesterone-like substance levels of female *Mictyris brevidactylus*. Bull. Inst. Zool., Acad. Sinica **32:** 221-228.

Shih JT. 1997. Sex steroid-like substances in the ovaries, hepatopancreases, and body fluid of female *Mictyris brevidactylus*. Zool. Stud. **36:** 136-145.

Shih JT, HJ Huang, IM Tso. 1989. The study of oxygen consumption of hepatopancreas of *Uca arcuata* (De Haan) *in vitro*. Biol. Bull. NTNU **24:** 33-39.

Shih JT, PS Leung, WC Hu. 1990. High pressure liquid chromatographic study of sex steroids of the fiddler crab, *Uca arcuata* (De Haan, 1835). Chinese Bioscience **33:** 1-8.

Shih JT, KY Lue, CH Wang. 1991. Crab fauna and the activities of ten crab species in Tanshui mangrove swamp of Taiwan. Ann. Taiwan Mus. **34:** 121-140. (in Chinese).

Shih JT, YM Wang. 1993. Progesterone-like substance in the ovaries, hepatopancreases, and hemolymph of female *Uca arcuata*. Bull. Inst. Zool., Acad. Sinica **32:** 12-126.

Skinner DM. 1985. Molting and regeneration. *In* DE Bliss, LH Mantel, eds. The biology of the crustacea. Vol. 9. New York: Academic Press, pp. 43-146.

Teshima SI, A Kanazawa. 1971. Bioconversion of progesterone by the ovaries of the crab, *Portunus trituberculatus*. Gen. Comp. Endocrinol. **17:** 152-157.

Van Den Oord A. 1964. The absence of cholesterol synthesis in crab, *Cancer paragus* L. Comp. Biochem. Physiol. **13:** 461-467.

Watson RD, E Spaziani. 1985. Effects of eyestalk removal on cholesterol uptake and ecdysone secretion by crab (*Cancer antennarius*) Y-organs *in vitro*. Gen. Comp. Endocrinol. **57:** 360-370.

Yano I. 1987. Effect of 17-beta-hydroxyprogesterone on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. Aquaculture **61:** 49-57.

Zandae DI. 1967. Absence of cholesterol synthesis as contrasted with the presence of fatty acid synthesis in some arthropods. Comp. Biochem. Physiol. **20:** 811-822.

Zoological Studies **37**(2): 102-110 (1998)

離體培養之短趾和尚蟹組織能轉化膽固醇為類固醇性激素

史金燾[1]　廖欽峰[2]

本研究是探討短趾和尚蟹之組織是否可以生合成類固醇性激素。和尚蟹之肝胰臟及卵巢在有同位素標誌的膽固醇加入的培養液中，經過 24 小時離體培養後，由組織及培養液中抽取類固醇，再經高效色層分析法鑑定其性質及含量。結果為:(1)肝胰臟及卵巢均能吸取培養液中有同位素標誌的膽固醇；(2)HPLC 分離出的類固醇性激素具有同位素的標誌，此等物質可能是新合成者；(3)生殖前期之末和生殖期和尚蟹組織生合成類固醇性激素的能力很低。

關鍵詞:蟹類，卵巢，肝胰臟，類固醇性激素。

[1]國立臺灣師範大學生物學系
[2]中央研究院動物研究所