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| journal or publication title | 鹿児島大学水産学部紀要=Memoirs of Faculty of Fisheries Kagoshima University |
| volume | 30 |
| page range | 349-356 |
| 別言語のタイトル | 海産無脊椎動物の生産する生理活性物質検索におけるウナギ肝浮遊細胞の利用 |
| URL | http://hdl.handle.net/10232/13241 |

Mem. Fac. Fish., Kagoshima Univ.
Vol. 30 pp. 349~356 (1981)

# Availability of Isolated Cells of the Eel Liver for Investigating Biologically Active Substances Produced by Marine Invertebrates

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**Abstract**

To investigate biologically active sustances produced by marine invertebrates isolated cells of the eel liver were used. Effect of extracts of tissues on glucose release from the liver cells of the eel and on gluconeogenesis in the liver cells were examined. Extracts of eye-stalk, mit-gut gland, and abdominal ganglion of prawn had stimuratory effect on glucose release. Extracts of tissues of sea cucumber had inhibitory effect on glucose release and gluconeogenesis. Gluconeogenesis in the eel liver cells was inhibited almost completely by the extract of digestive organ of starfish.

The effect of extracts of tissues of marine invertebrates on glucose release from the liver cells of the eel (*Anguilla japonica*) and on gluconeogenesis in the eel liver was investigated. It is known that Gastropoda and Bivalvia of Mollusca have neuroendocrine system[1] and Cephalopoda has some endocrine gland[2]. However, there is little report on the metabolism of carbohydrate and amino acid in these Mollusca. So no physiological effect of neuroendocrine and endocrine system on these metabolism has known. Crustacea has endocrine and neuroendocrine system and physiological function of these system has been well studied. For example, it is known that there is hormones regulating the metabolism of carbohydrate and protein[3] and hyperglycemic hormone[4] secreted from eyestalk.

To use isolated cells of the eel liver is not necessarilly good method to inverstigate the presence or absence of hormones or biologically active substance in tissues of invertebrates. However, since we can prepare rather easily cell-suspension of the eel liver and determine gluconeogenesis and glycogenolysis in the cells very exactly, we examined the effect of extracts of each tissues of marine invertebrate. Prawn, sea hares, sea cucumber, ligia, sea urchin, starfish, and sea squirts were used. Extracts of kidney, pancreas, gill, brain, and pituitary of the eel were also investigated.

## Experimental Procedures

**Isolation of liver cells of the eel** Japanese cultured eels weighing about 150 g

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were purchased from the fish market. They were kept in freshwater aquaria and fasted for 3 to 14 days before use.

Livers were excised from anethesized eels and perfused with modified Krebs-Ringer bicarbonate buffer (pH 7.4) containing collagenase (15 mg per 50 m*l*). The procedures of the perfusion of liver and the isolation of liver cells have been described previously[5,6].

**Measurement of the incorporation of $^{14}C$-labelled substrates into glucose and determination of glucose** Eel liver contains glycogen even after fasting for several months as reported by Dave *et al*[7]. Similarly, glucose was released to the incubation mixture from liver cells prepared from fasted eels[6]. Therefore, labelled substrates were used for examining gluconeogenesis in liver cells. L-[U-$^{14}C$]-Alanine (50 $\mu$Ci), [2-$^{14}C$]-pyruvate (50 $\mu$Ci) and DL-[2-$^{14}C$]-lactate were dissolved in 4 m*l* of 0.5 M unlabelled substrate. L-[U-$^{14}C$]-Alanine was taken to dryness by storage in vacuo over $P_2O_5$ for overnight to remove the ethanol before use.

The reaction mixture for gluconeogenesis contained, in a final volume of 2 m*l*, one m*l* of cell suspension, 20 $\mu l$ of $^{14}C$-labelled substrate, 0.1 m*l* of extract and Ringer solution gassed with 95% $O_2$ and 5% $CO_2$. The mixture was incubated at 30°C for 30 to 60 min. The reaction was initiated by the addition of cell suspension preincubated at 30°C for 15 min and stopped by the addition of 0.2 m*l* of 60% $HClO_4$. After centrifugation at 3,000 rpm for 10 min, the supernatant was neutralized with 0.5 m*l* of 2 M $K_2CO_3$ and cooled. One m*l* of neutralized supernatant was applied to a Dowex 1X8 column (0.6×5 cm, $HCOO^-$ form, 100–200 mesh) and Dowex 50X8 column (0.6×2 cm, $H^+$ form, 200–400 mesh). 0.5 m*l* of $^{14}C$-glucose fraction was added to 5 m*l* of toluene containing 4 g of 2, 5-diphenyloxazole (DPO), 0.1 g of 1, 4-bis (2-(5-phenyloxazoyl))benzene (POPOP) and 500 m*l* of Triton X–100 per litre. The radioactivity was measured using a Beckman LS–230 spectrophotometer.

The reaction mixture for glucose release from eel liver-cells contained, in a final volume of 2 m*l*, one m*l* of cell suspension, 0.1 m*l* of extract and Ringer solution gassed with 95% $O_2$ and 5% $CO_2$. The neutralized supernatant obtained by the procedures described above was used for determination of glucose by colorimetirc[8] and enzymatic methods[9].

**Preparation of extract** Each tissue was extracted by 4 vol of water or 4 vol of 6% $HClO_4$. After tissue was homogenized with ice water by Ultra Tarrax (Type 10N), homogenate was centrifuged at 20,000 rpm for 1 h at 5°C. Supernatant was used for experiments. Homogenate of tissue by 6% $HClO_4$ was centrifuged at 3,000 rpm for 10 min and the supernatant was neutralized with 2 M $K_2CO_3$. Neutralized supernatant was used for experiments.

When a small tissue such as abdominal ganglion of prawn or pituitary of eel was homogenized, 10 to 20 vol of water or 6% $HClO_4$ was used. Tissue extract was expressed as m*l* per g tissue.

**Chemicals and enzymes** L-[U-$^{14}$C]-Alanine, [2-$^{14}$C]-pyruvate, and DL-[2-$^{14}$C]-lactate was purchased from RCC Amersham. ATP and NADP were obtained from Oriental Yeast Co. Collagenase from *Cl. histolyticum* was obtained from P–L Biochemicals and glucose-6-phosphate dehydrogenase (from yeast) and hexokinase (from yeast) were from Boehringer Mannheim. Other chemicals were purchased from Wako Chemicals.

## Results and discussion

**Effects of extracts on glucose release from eel liver-cells** As shown in Table 1. and Fig. 1, extracts of eyestalk, mit-gut gland, and abdomianal ganglion of

Table 1. Effect of extracts on glucose release from liver cells of the eel.

| | Organism Tissue | Extract ml/g tissue | Glucose (mg/g wet wt. h. at 30°C) Colorimetric Method | Enzymatic Method |
|---|---|---|---|---|
| | Sea hares | | | |
| | Control | | 10.31±2.18 | 8.10±0.78 |
| | Digestive organ | 3.33 | 6.38±1.14 | 6.63±0.95 |
| | Purple gland | 4.00 | 10.06±0.95 | 8.35±0.80 |
| | Abdominal ganglion | 16.67 | 8.84 | 8.59±0.78 |
| | Sea cucumber | | | |
| | Control | | 10.31±2.18 | 8.10±0.78 |
| Extracts | Digestive organ | 3.33 | 1.23±0.27* | 1.47±0.28* |
| by water | Gonad | 3.33 | 6.14±0.61 | 4.91±0.86** |
| | Radial canal | 4.00 | 1.23±0.27* | 1.96±0.19* |
| | Eel | | | |
| | Control | | 2.85±0.60 | 3.81±0.82 |
| | Gill | 3.84 | 6.08±1.26* | 6.43 |
| | Kidney | 3.84 | 5.36 | 6.79±1.54* |
| | Pancreas | 5.17 | 2.85±0.60 | 3.81±0.86 |
| | Brain | 22.10 | 6.31±1.31* | 6.31±1.31* |
| | Pituitary | 600 | 5.72±1.18 | 6.67±1.42* |
| | Prawn | | | |
| | Control | | 2.85±0.60 | 3.81±0.82 |
| | Eyestalk | 3.03 | 6.67±1.39* | 8.22±1.71** |
| | Mit-gut gland | 3.79 | 6.91±1.41* | 7.74±1.63 |
| | Abdominal ganglion | 13.49 | 6.08±1.26* | 6.79±2.76 |
| Extracts | Eel | | | |
| by 6% $HClO_4$ | Control | | 6.25±1.32 | 3.84 |
| | Gill | 3.9 | 4.55±0.97* | 2.70 |
| | Kidney | 3.9 | 5.68±1.23 | 3.20 |
| | Pancreas | 5.2 | 2.27±0.64* | 2.63 |
| | Brain | 22.5 | 5.12±1.12** | 3.20 |
| | Pituitary | 600 | 6.54±1.38 | 3.48 |

* $P<0.01$ ** $P<0.05$

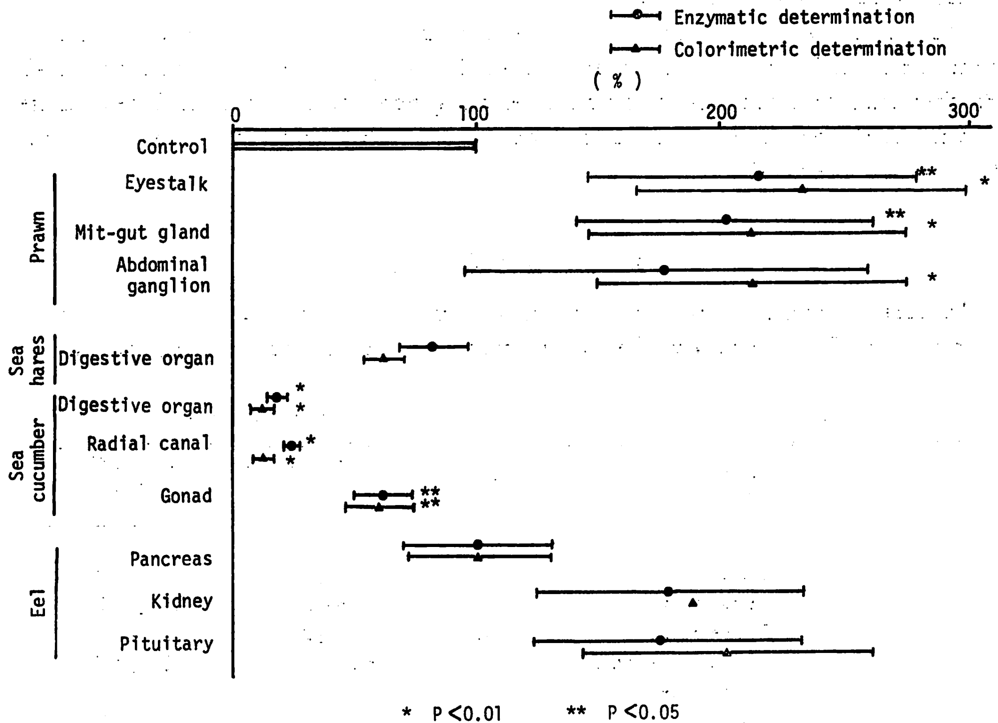


**Fig. 1.** Effect of extracts on glucose release from isolated cells of the eel liver. Tissues of prawn were extraxted by 6% $HClo_4$. Others were extracted by water.

prawn by 6% $HC10_4$ had an effect of increasing glucose release from liver cells. These extracts stimulates glucose release by 2 times. Inhibitory effect of water-extract of digestive organ and radial canal of sea cucumber was observed. They inhibited glucose release by 80 to 90%. Water-extracts of gill and brain of the eel had stimuratory effect, but extracts of gill, brain and pancreas of the eel by 6% $HC10_4$ had almost no effect. Since extracts of gill, brain and pancreas by 6% $HC10_4$ had almost no effect on glucose release and extracts by water had stimuratory effect, these effectors in extracts seemed to be something like protein.

As Kleinholz *et al*[4)] reported that hyperglycemic hormones from eyestalk were peptides, there are some possibility that the extract of eyestalk having stimulating effect on glucose release contained some peptides like hyperglycemic hormones. Simiraly there were some substances to stimulate glucose release from eel liver-cells in extracts of mit-gut gland and abdominal ganglion. It is unknown what kind of substances they are.

It was found that the extracts of sea cucumber had inhibitory effect on glucose release. This inhibitory effect may be due to some peptides like insulin, because there are some reports describing the presence of peptide like insulin in marine

invertebrate[10]. However, there is another possibility that this inhibitory effect is due to saponin[11].

**Effect of extracts on gluconeogenesis** Extracts of eyestalk, mit-gut gland, and abdominal ganglion of prawn by 6% $HClO_4$ have stimulatory effect on gluconeogenesis from $^{14}C$-pyruvate in the eel liver-cells as shown in Fig. 2. Water extracts of digestive organ and radial canal of sea cucumber, and of digestive organ of sea urchin have inhibitory effect on gluconeogenesis from $^{14}C$-lactate, $^{14}C$-pyruvate, and $^{14}C$-alanine. Extract of digestive organ of starfishes inhibited gluconeogenesis almost completely (Table 2).

Table 2. Effect of extracts by water on gluconeogenesis in the eel liver.

| Organism Tissue | Extract ml/g tissue | Gluconeogenesis (umole/g wet wt. h. at 30°C) Substrate | | |
|---|---|---|---|---|
| | | Lactate | Pyruvate | Alanine |
| Prawn | | | | |
| Control | | 39.6±5.8 | | |
| Eyestalk | 8.21 | 41.6±5.9 | | |
| Mit-gut gland | 3.83 | 40.6±5.8 | | |
| Abdominal ganglion | 19.88 | 38.0±6.7 | | |
| Ligia | | | | |
| Control | | 56.1±6.9 | 35.2±6.4 | |
| Digestive organ | 22.49 | 40.3±5.3 | 34.5±4.6 | |
| Abdominal ganglion | 4.76 | 49.0±6.2 | 42.7±5.5 | |
| Sea hares | | | | |
| Control | | 45.3±5.3 | 29.3±3.3 | 25.2±2.9 |
| Digestive organ | 3.33 | 33.4±4.1 | 20.5±5.3 | 20.8±3.9 |
| Purple gland | 4.00 | 43.0±7.2 | 31.4±5.6 | 24.9±3.1 |
| Abdominal ganglion | 16.67 | 46.7±6.2 | 33.8±5.2 | 24.4±2.8 |
| Sea cucumber | | | | |
| Control | | 45.3±5.3 | 29.3±3.3 | 25.0±2.9 |
| Digestive organ | 3.33 | 7.5±3.4* | 0.8±0.2* | 0.6±0.2* |
| Gonad | 3.33 | 45.5±7.6 | 33.5±6.3 | 34.4±3.9 |
| Radial canal | 4.00 | 37.7±5.3 | 15.6±2.7** | 5.6±0.9* |
| Sea urchin | | | | |
| Control | | 45.3±5.3 | | 25.0±2.9 |
| Digestive organ | 4.00 | 28.8±4.2 | | 8.7±1.4* |
| Starfish | | | | |
| Control | | 22.6±1.8 | | 25.0±2.9 |
| Digestive organ | 4.00 | 1.1±0.4* | | 0.2±0* |
| Sea squirt | | | | |
| Control | | | | 25.0±2.9 |
| Digestive organ | 4.00 | | | 30.5±4.7 |

* $P<0.01$ ** $P<0.05$

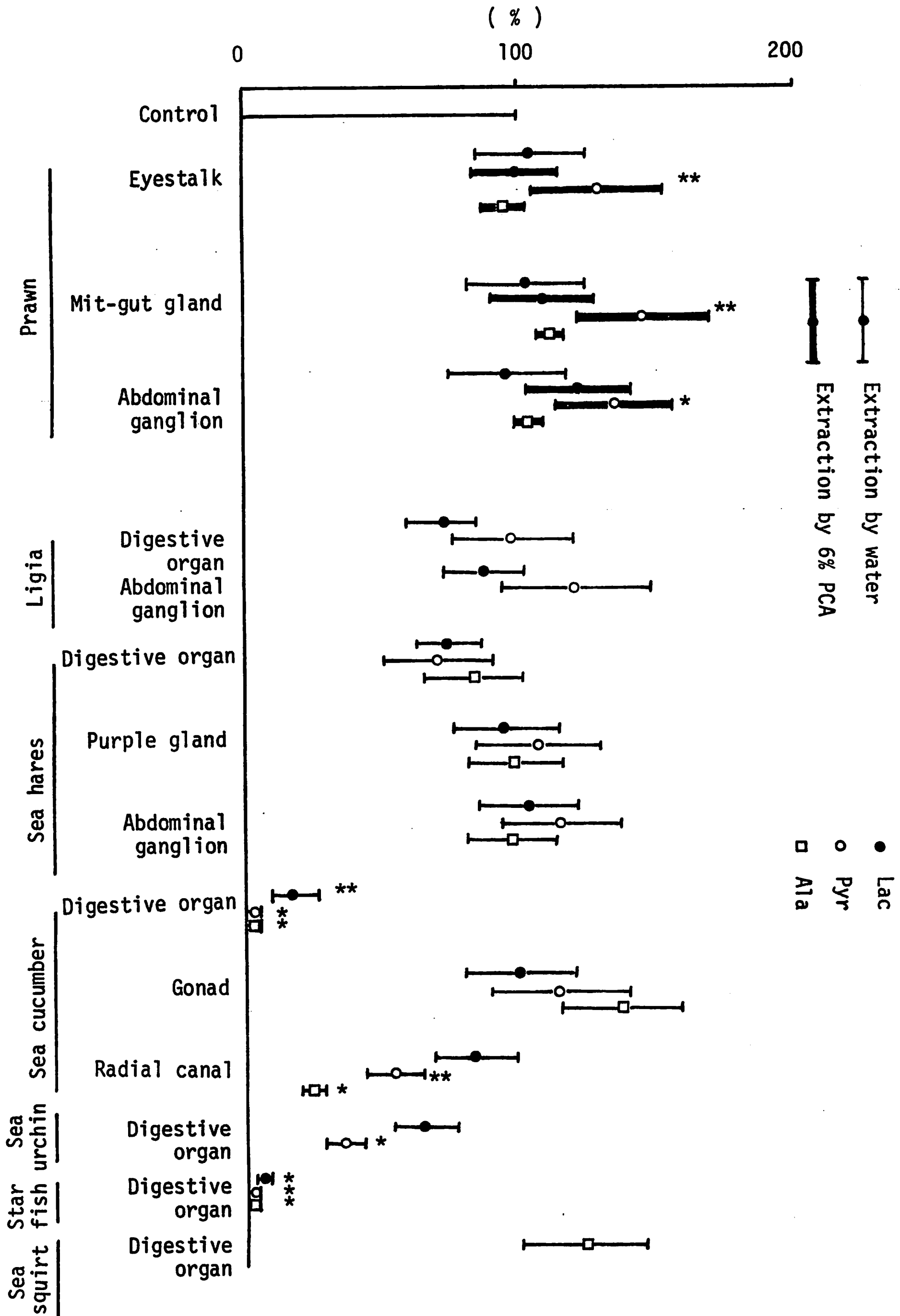


**Fig. 2.** Effect of extracts on gluconeogenesis.

It is interesting that the extracts of eyestalk, mit-gut gland, and abdominal gland had stimuratory effect on gluconeogenesis, since there was no reports that whether hyperglycemic hormones in crustaceas stimulated gluconeogenesis or did not[10]. These extracts stimulated glucose release from liver cells of the eel as well as gluconeogenesis in the eel liver.

Yasumoto *et al*[12] reported the presence of saponin in starfishes. Saponin has hemolytic toxin[11]. Saponin seems to react with cell membrane and to inhibit metabolism in cell. The extract into which 6% of $HC10_4$ was added still had inhibitory effect on gluconeogenesis by 49%. Similary the extract treated at 60°C for 30 min still had inhibitory effect by 66%. After the gel-filtration by Sephadex G-75, fractions monitored at 244 nm had inhibitory effect. It is known that saponin absorbs the light at 244 nm. Though the substance in the extract of starfish, which inhibited gluconeogenesis almost completely, was not identified as saponin, it is thought this inhibitory effect was due to saponin. Simiraly inhibitory effect of the extracts from sea cucumber seems due to saponin.

## Acknowledgement

We wish to thank Dr. H. Hirata, S. Kadowaki, T. Nakazono, and T. Kasedo, the Fisheries Research Laboratory, Kagoshima University, for their help to take samples.

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