NOTE
Effects of Hypophysectomy and Salinity Change on Plasma Cortisol Concentration in the Japanese Eel, Anguilla japonica

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Synopsis
The amount of eel plasma cortisol was determined by the method of Van der Vies (1961) with some modification. The plasma cortisol level in the intact eels kept in either freshwater or seawater was about 4 μg/100 ml. Single intraperitoneal injection of ACTH caused a marked increase in the level of this substance after 2 hrs. In the hypophysectomized eel kept for ten days in freshwater, the cortisol concentration decreased to 0.3 μg/100 ml. Transfer of the intact eel from freshwater to seawater resulted in a significant increase in its concentration within 2 hrs., whereas significant increase was not observed when the eel was retransferred from seawater to freshwater. Moreover, the increase after transfer to seawater was not observed when the eel had previously been hypophysectomized. These data suggest that interrenal of the eel is under direct control of pituitary. Possible involvement of cortisol in the adaptation mechanism to seawater in the eel was discussed.

Recently some evidence has been advanced to indicate that pituitary-interrenal axis of the eel is involved in the adaptation mechanism to seawater, especially with respect to the intestinal water absorption and also to the sodium efflux at the gill (Hirano, 1967; Hirano et al., 1967; Hirano and Utida, 1968; Mayer et al., 1967; Maetz, 1967). By the incubation study in vitro, cortisol has been shown to be the major corticosteroid secreted by the interrenal of the eel (Butler, 1965; Leloup-Hatey, 1966; Sandor et al., 1967). However, simple fluorometric methods which are commonly applied for rat and human plasma, provided to be grossly inaccurate in the determination of circulating cortisol in the eel, possibly because of higher lipid content of the eel plasma as compared with the mammalian plasma. In order to remove these contaminants, washing of the plasma with tetrachloromethane seemed to be effective. Therefore the procedure of Van der Vies (1961) was modified for the estimation of plasma cortisol of the eel. The present report deals with the simple fluorometric method which has been successfully used to investigate the pituitary-interrenal axis of the eel with special reference to seawater adaptation.

Materials and Methods
Cultured Japanese eel, Anguilla japonica, were purchased from a commercial source and used as material. These fish, weighing from 130 to 150 g, were maintained in a freshwater aquarium at 20°C for 1 week before use. The ionic concentrations of freshwater and seawater used have been described by Oide and Utida (1967). In some of the intact eels in freshwater, ACTH (Schering) was injected intraperitoneally at a dose of 1 U. Hypophysectomy was carried out in the same manner as described previously (Hirano et al., 1967).

For the determination of cortisol of the eel plasma, the fluorometric method of Van der Vies (1961) was modified. The main procedure developed for the eel was as follows: After anesthetizing the eel with 0.1% solution of MS 222, blood sample was collected with
Fig. 1. Rate of development of fluorescence of cortisol and eel plasma.

Fig. 2. Fluorescence spectra of cortisol and eel plasma (cf. Fig. 4). Exciting wave length, 436 μm.

siliconized syringe from the ventral aorta and immediately centrifuged at 10,000 rpm for 5 min. at 0°C. One ml of plasma thus obtained was diluted to 2 ml with distilled water, and extracted with 8 ml of dichloromethane by shaking vigorously for 1 min. After centrifugation for 5 min. at 3,000 rpm, 6.5 ml of the solvent phase was evaporated to dryness under reduced pressure at a temperature not exceeding 37°C. To the dry residue was added 2.5 ml of distilled water, then 20 ml of tetrachloromethane, and the tube was shaken for 1 min. After centrifugation tetrachloromethane layer was discarded. The remaining water phase was again shaken with 20 ml of tetrachloromethane, centrifuged and organic phase was discarded. A 2 ml aliquot of water phase was extracted with 5 ml of dichloromethane by shaking for 1 min. After removing the water phase, 1 ml of 0.1 N NaOH was added, the tube was shaken for 15 sec. and centrifuged. The alkaline wash was discarded. To the organic phase 4 ml of the fluorescence reagent (75% sulphuric acid-25% ethanol, v/v) was added, shaken for 1 min. and centrifuged.

Since the major part of the fluorescence in the standard and plasma developed within 30 min. (Fig. 1), the fluorescence was read 30 min. after the addition of the fluorescence reagent in a Hitachi 203 spectrofluorometer with an exciting wave length of 436 μm and an emitted wave length of 530 μm. Standards containing 0.1 and 0.2 μg of cortisol, and a blank consisting of 2 ml of water were carried through the same procedure. Fluorescence spectrum of the eel plasma thus obtained was in good agreement with that of standard cortisol (Fig. 2). Moreover, the experiments in which cortisol was determined in various volumes of plasma indicated that there was very little residual fluorescence at zero volume, indicating high specificity (Fig. 3).

Results

As shown in Table 1, mean plasma cortisol concentration was 3.8 μg/100 ml in the intact eel in freshwater. This value increased markedly 2 hrs. after the intraperitoneal injection of ACTH. Two hours after transferring the eel from freshwater to seawater, the plasma cortisol increased to 7.8 μg/100 ml. However, after 2 months of adaptation to seawater, it returned to initial freshwater level. On the other hand,
Table 1. Effect of hypophysectomy and salinity change on plasma cortisol level of the eel

<table>
<thead>
<tr>
<th>Eel</th>
<th>Treatment</th>
<th>No. of eel</th>
<th>Plasma cortisol (µg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Freshwater (FW)</td>
<td>5</td>
<td>3.8 ± 0.9*</td>
</tr>
<tr>
<td>Intact</td>
<td>FW, ACTH (IU)</td>
<td>5</td>
<td>21.9 ± 1.8**</td>
</tr>
<tr>
<td>Intact</td>
<td>FW-Seawater (SW)</td>
<td>2 hrs.</td>
<td>7.8 ± 1.0***</td>
</tr>
<tr>
<td>Intact</td>
<td>SW</td>
<td>2 months</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>Intact</td>
<td>SW (2 months)-FW</td>
<td>2 hrs.</td>
<td>6.0 ± 2.9</td>
</tr>
<tr>
<td>Hypox</td>
<td>FW</td>
<td>10 days</td>
<td>0.28 ± 0.17**</td>
</tr>
<tr>
<td>Hypox</td>
<td>FW (10 days)-SW</td>
<td>2 hrs.</td>
<td>0.19 ± 0.19**</td>
</tr>
</tbody>
</table>

* : Mean ± standard error.
**,**,**,**: significantly different from the intact freshwater eel at P<0.001 and P<0.02, respectively.

transfer of the seawater adapted eel to freshwater did not result in significant increase in the amount of the plasma cortisol.

When the eels kept in freshwater were hypophysectomized and placed in freshwater for 10 days, only negligible amount of cortisol was detected. When these hypophysectomized eels were transferred to seawater, no increase in the plasma cortisol was observed after 2 hrs.

Discussion

Leloup-Hatey (1964) measured plasma 17-OH corticosteroids in the eel following the procedure of Nelson and Samuels (1952). This colorimetric method, measuring Poter-Silber chromogens, involves chromatography and requires fairly large plasma samples. In the Japanese cultured eel as used in the present study, only small volumes are available for analysis. On the other hand, simple fluorometric methods which omitted chromatographic purification are highly sensitive, however, are not applicable to the eel owing to a large amount of contaminants in the plasma (Fig. 4). Most of the interfering fluorogens seem to be lipid in nature, since the fluorescence spectra of the glycerides resemble with that of the eel plasma without tetrachloromethane extraction. In order to remove these contaminants, initial petroleum ether washing of the plasma as suggested by Van der Vies (1961) did not influence specificity hence omitted from the present procedure, but a partition between water and tetrachloromethane was very effective (Fig. 2). Cortisol concentration as determined by the present method was about 4 µg/100 ml in the intact eels both in freshwater and in seawater. On the other hand, the apparent cortisol level of the same plasma was about 70 µg/100 ml, when the tetrachloromethane partition was omitted (Fig. 4). This resting level of cortisol was in good agreement with that of 17-OH corticosteroid of A. anguilla as determined by phenylhydrazine sulfuric acid reaction (Leloup-Hatey, 1964), and with the value obtained in A. rostrata using similar fluorometric method (Butler et al., 1969a, b). Thus, the present modification of Van der
Vies's procedure offers a simple technic for the estimation of circulating cortisol in the eel plasma.

The presence of a pituitary-interrenal axis of the eel in freshwater has been well studied histologically after hypophysectomy and replacement therapy (Fontaine and Hatey, 1953; Hatey, 1954a, b; Olivereau and Fromentin, 1954; Olivereau, 1965). Leloup-Hatey (1964) demonstrated that plasma 17-OH corticosteroids of A. anguilla increased within 4 hrs. after ACTH injection. Butler et al. (1969a, b) reported a marked decrease in the plasma cortisol concentration after hypophysectomy and interrenallectomy of A. rostrata in freshwater, and an increase following 10 daily injections of ACTH into the hypophysectomized eel. In the present study, the plasma cortisol in A. japonica increased markedly 2 hrs. after the single injection of ACTH, and increase in the plasma cortisol following transfer of the eel to seawater was not observed after hypophysectomy. These data strongly suggest that the release of cortisol from the internal of the eel is under direct control of the pituitary corticotrophin.

As reported previously, the hypophysectomized eel treated with cortisol in seawater has been shown to restore the high rate of water movement in the isolated intestine similar to that observed in the intact fish in seawater (Hirano, 1967). Moreover, specific augmentative action of ACTH and cortisol was also observed in the isolated intestines of the freshwater eel (Hirano and Utida, 1968). In the isotope study of ion exchange mechanism in the gill of A. anguilla, Mayer et al. (1967) and Maetz (1967) reported that hypophysectomy or interrenallectomy reduced the sodium turnover rate in the gill of seawater adapted eel, and ACTH or cortisol restored the normal pattern of sodium exchange. These data suggest that ACTH-cortisol system in the eel is involved in the adaptation mechanism to seawater by acting at the intestine and the gill. The present observations that transfer of the eel from freshwater to seawater resulted in a significant increase in cortisol concentration, and that significant increase was not observed when the eels were retransferred from seawater to freshwater seem to support the involvement of this system in salt adaptation of the eel.

References