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SYNTHESIS AND RELEASE OF PHOSPHOPROTEIN AND PHOSPHOLIPID IN THE FEMALE FRESHWATER CATFISH, *HETEROPNEUSTES FOSSILIS* IN RESPONSE TO ESTRADIOL-17β DURING TWO DIFFERENT PHASES OF ANNUAL REPRODUCTIVE CYCLE

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Ajay Kumar Singh (1992) Synthesis and release of phosphoprotein and phospholipid in the female freshwater catfish, *Heteropneustes fossilis* in response to estradiol-17β during two different phases of annual reproductive cycle. *Bull. Inst. Zool., Academia Sinica* **31**(1): 23-32. Changes in phosphoprotein and phospholipid of the liver and serum were observed in response to the administration of different doses of estradiol-17β during postspawning and preparatory phases of the reproductive cycle of *Heteropneustes fossilis*. All tested doses of estradiol-17β (5 μg, 10 μg, and 50 μg/fish for 7 days) increased liver RNA without altering DNA content in both postspawnig and preparatory phases, only medium (10 μg/fish) and high (50 μg/fish) doses during the postspawning phase, and all three doses (5 μg, 10 μg and 50 μg) during the preparatory phase heightened phospholipid and phosphoprotein levels in both the liver and serum. Treatment with estradiol-17β during both reproductive phases at doses of 10 and 50 μg/fish resulted in increased liver protein. The influences of estrogen treatment on phospholipid synthesis varied slightly according to individual phases of the reproductive cycle. Low doses of estradiol-17β (5 μg/fish) induced the synthesis of lipids other than phospholipid in the liver during the postspawning season. On the other hand, medium (10 μg/fish) and high (50 μg/fish) doses of the same hormone triggered the synthesis of phospholipid without affecting total lipid concentrations in the liver during preparatory phase.

Key words: DNA, Estradiol-17β, Lipid phosphorus, Protein phosphorus, RNA.

Vitellogenin, a female-specific yolk precursor, has been identified as a lipophoprotein in amphibia (Follet *et al.*, 1968; Wallace and Jared, 1969; Wallace and Bergink, 1974), reptiles (Yaron and Widzer, 1978; Sapp *et al.*, 1979) and fish (Craik, 1979a; Crim and Idler, 1978). Except for *Xenopus leavis* (Redshaw and Follet, 1971), comparativelly little attempt has been made to study the detailed chemical characteristics of vitellogenin in lower vertebrates. However, in fish, vitellogenic response to estrogen was studied indirectly by measuring biochemical constituents associated with vitellogenesis. For example, estrogen treatment increased liver size, total protein, total lipid, phospholipid, and phosphoprotein of the liver and serum in several species of

fish (Campbell and Idler, 1976; De Vlaming *et al.* 1977a, 1977b; Craick, 1978b; Hori *et al.*, 1979), including hagfish (Yu *et al.*, 1981). Histological investigations also revealed that injections of estradiol-17β caused increases in cytoplasmic vacuolization (lipid material) of the liver in intact male and female eels (Olivereau and Olivereau, 1979a, 1979b). It has also been suggested that synthesis of a yolk precursor in the liver and its release into the blood is brought about by estrogen, whereas mobilization of a yolk precursor from the blood to the ovaries is under the control of pituitary gonadotropin (Campbell and Idler, 1976; Nath and Sundararaj, 1981; Sundararaj and Nath, 1981). The synthesis and release of estrogen has already been demonstrated in fish ovaries (Nagahama *et al.*, 1976; Yaron *et al.*, 1977).

A survey of the literature indicates that, in teleosts, apparently no attempt has been made to measure the RNA and DNA content of the liver in response to estrogen. When investigating the involvement of estrogen in vitellogenesis, it becomes essential to study levels of RNA in relation to DNA, as it would help to define the role of estrogen at the transcription level.

This experiment described the effects of estradiol-17β on the synthesis and release of phosphoprotein and phospholipid in freshwater catfish, *Heteropneustes fossilis*, during the postspawning and preparatory phases of its reproductive cycle.

MATERIALS AND METHODS

Adult *Heteropneustes fossilis* famales with average weights of 35-40 g and lengths of 18.5-20 cm were collected from Ramgarh Lake near Gorakhpour (India). Two separate experiments were conducted in October and February, during the postspawning and preparatory phases of the fish's annual reproductive cycle. The various phases of that cycle as described by Singh and Singh (1979) were incorporated. Fish were kept under laboratory conditions for one week, and were fed minced liver; specimens were not fed during hormone treatment. Aquaria temperatures were not controlled; however, variation in temperature was similar to that of control and experimental aquaria, ranging from 26 to 28°C in October, and 21 to 23°C in February. Daily photoperiods were measured at 11.5 L/12.5 D in October, and 10.5 L/13.5 D in February.

Hormone administration

Estradiol-17β was homogenized in a solution of 0.6% NaCl with glass homogenizer, plus a drop of tween 80. Tween 80 was also added to the control saline. Before each phase of the experiment, specimens were divided into four batches. Batch 1 individuals were injected (*i.p.*) with the 0.6% saline, whereas batch 2, 3 and 4 specimens were injected with three different doses of estradiol-17β. The fish were injected daily for 7 days. Details on treatment, dosages, and number of fish in each batch are shown in Tables 1-4. Carrier fluid volume was maintained at 0.2 ml, and the fish were mildly anaesthetized (1:4,000, MS 222) before each injection. A two-way analysis of variance (Sokol and Rohlf, 1969) was used to calculate the significance of difference among estrogen responses during postspawning and preparatory phases. Student's *t* test was used to compare differences between batches in the experimental groups.

Collection of liver samples and blood serum

Fish were sacrificed by severing heads 24 hours after final injections. Blood was collected by using heparinized

syringes, then centrifuged at 3,500 RPM for 10 minutes. Sera obtained from individual fish were collected and frozen prior to analysis. Livers were dissected, washed with 0.6% saline, blotted to remove excess saline, weighed to the nearest 0.1 mg with a single pan electric balance, and stored in freezers.

Determinations

Liver were divided into equal halves. For each liver one half was used for estimating total lipid and lipid phosphorus content, and the other half was utilized for estimating RNA, DNA, protein, and protein-bound phosphorus. Liver lipid was extracted using chloroform-methanol (Folch *et al.*, 1957); details of this procedure have already been described (Singh, 1979, 1981). Extracted lipid was dissolved in chloroform, and its phosphorus content was measured by the Fiske and Subba Row (1925) method. RNA, DNA, and protein were fractionated from the other liver halves using the Schmidt-Thannhouser method (Munro and Fleck, 1966). Weighed amount of liver were homogenized with 10% TCA. After centrifugation (3,500 RPM for 15 minutes), residue was retained, and supernatant discarded. The process was then repeated using 5% TCA. Precipitate was washed twice with a chloroform and methanol solution (2:1), then with ether to remove the lipid material. For RNA hydrolysis, precipitated material was suspended in 0.3 N KOH at 37°C for 1 hour, then cooled to 0°C. DNA and protein were both precipitated by acidifying the alkali digest with 10% TCA. After centrifugation, the supernatant was conserved as an RNA fraction, and pellets were incubated with 10% TCA at 80°C for 15 minutes, then cooled to 0°C. This suspension was centrifuged, and the supernatant was collected as a DNA fraction. Precipitate was digested in 1N NaOH for protein estimation.

For estimating serum protein, lipid phosphorus, and protein phosphorus, 0.2 ml of serum was treated with twice its volume of 10% TCA; precipitate was then washed with the same volume of 5% TCA. Pellets obtained were then subjected to the same treatment described above to get a different fraction. Here, the lipid extract (chloroform+methanol and ether extract) was also saved for lipid phosphorus estimation. RNA and DNA fractions were discarded, and only protein residue was retained for measuring protein and its phosphorus content. Protein phosphorus was measured by the same method as described earlier for measuring lipid phosphorus. RNA and DNA were measured by orcinol and diphenylamine reagents, respectively (Schneider, 1957), utilizing yeast RNA and calf thymus DNA as standards. Protein was estimated by methods established by Lowry *et al.* (1951).

RESULTS

1. Postspawning phase

Treatments with different doses of estradiol-17β (5 μg, 10 μg, and 50 μg/fish) over a 7-day period increased liver RNA during the postspawning phase (Batches 2-4, Table 1) when compared with the saline-treated control fish. However, liver DNA remained unchanged. Only medium (10 μg/fish) and high (50 μg/fish) doses of estradiol-17β stimulated the synthesis of phosphoprotein as made evident by heightened levels of protein and protein phosphorus (Batches 3 and 4, Table 1). Although all doses of estradiol-17β increased total liver lipid (Batches 2-4, Table 1), only medium (10 μg/fish) doses of the hormone elevated lipid phosphorus (Batches 2 and 3, Table 1). This suggests that low doses of estradiol-17β stimulate the synthesis of liver lipid

Table 1
Effects of estradiol-17β (E_2) on RNA, DNA, total protein, lipid, protein phosphorus, and lipid phosphorus content in livers of *H. fossilis* during postspawning phase

Batch[a]	Treatment (daily for 7 days)	A RNA (mg/g wet weight)	B DNA (mg/g wet weight)	C Protein (mg/g wet weight)	D Protein phospho-rus (mg/g wet weight)	E Lipid (mg/g wet weight)	F Lipid phos-phorus (mg/g wet weight)
1- (6)	0.6% saline	4.60 ±0.01	2.90 ±0.09	96.00 ±4.35	5.30[b] ±0.09	54.00[c] ±4.41	10.40[d] ±0.85
2- (5)	E_2 5 μg/fish	6.80** ±0.12	3.20 ±0.08	97.00 ±5.65	5.40 ±0.11	66.00* ±5.43	10.40 ±0.96
3- (6)	E_2 10 μg/fish	8.60** ±0.15	3.30 ±0.15	115.00* ±5.71	11.20** ±0.95	71.00** ±4.37	20.60*** ±0.97
4- (6)	E_2 50 μg/fish	8.20** ±0.15	3.30 ±0.19	145.00*** ±7.32	12.50** ±0.88	75.00** ±6.11	21.00*** ±0.96

[a] No. of fish in each batch is given in parentheses.
Values are given as mean±SEM.
Significance of difference compared between Batch 1 and Batches 2-4 (*t* test); $*p<0.05$, $**p<0.01$, $***p<0.001$.
Significantly different from preparatory phase (two-way ANOVA); [b] $p<0.01$, [c,d] $p<0.05$.
Dose response relationship (two-way ANOVA). Column A, D, E, $p<0.05$; Column C, $p<0.01$; and for Column B and F, not significant.

Table 2
Effects of estradiol-17β (E_2) on total protein, lipid phosphorus, and protein phosphorus content in serum of *H. fossilis* during postspawning phase

Batch[a]	Treatment (daily for 7 days)	A Total protein (g/100 ml)	B Lipid phosphorus (mg/100 ml)	C Protein phosphorus (mg/100 ml)
1- (6)	0.6% saline (0.2 ml/fish)	3.50[b] ±0.45	12.30[c] ±0.55	3.20[d] ±0.20
2- (5)	E_2 5 μg/fish	4.20 ±0.55	13.70 ±0.60	4.20 ±0.35
3- (6)	E_2 10 μg/fish	8.10* ±0.68	20.30** ±1.15	8.20** ±0.51
4- (6)	E_2 50 μg/fish	8.20* ±0.66	20.50** ±1.02	10.30** ±0.62

[a] No. of fish in each batch in given in parentheses.
Values are given as mean±SEM.
Significance of difference compared between Batch 1 and Batches 2-4 (*t* test); $*p<0.01$, $**p<0.01$.
Significantly different from preparatory phase (two-way ANOVA); [b,c,d] $p<0.05$.
Dose response relationship (two-way ANOVA). Column B, $p<0.05$; Column A and C, not significant.

other than phospholipid. Only medium and high doses of estradiol-17β increased total protein, protein phosphorus, and lipid phosphorus (batches 3 and 4, Table 2) in serum, whereas low doses were found to be ineffective (batch 2, Table 2).

2. Preparatory phase

Similar to the postspawning phase, all three doses of estradiol-17β increased liver RNA (batches 2-4, Table 3) without altering liver DNA levels. A significant increase in liver protein was observed in response to hormone treatments at 10 and 50 μg/fish. However, all tested doses increased protein-bound phosphorus in livers (batches 2-4, Table 3). Only high doses (50 μg/fish) of the hormone showed increases in liver lipids (batch 4, Table 3); however, elevated levels of liver lipid phosphorus were encountered in response to all doses of estradiol-17β (batches 2-4, Table 3). It seems that low (5 μg/fish) and medium (10 μg/fish) doses of estrogen facilitate the synthesis of phospholipid at a cost to other types of lipid. Increases in total protein, protein phosphorus, and lipid phosphorus were noticed in serum after administering the three different doses of estradiol-17β (batches 2-4, Table 4).

3. Physiological statuses during two different phases and dose-response relationships

A two-way ANOVA revealed that RNA, DNA, protein, and lipid phosphorus in the livers were not significantly different during the postspawning and preparatory phases of the reproductive cycle. Fish livers showed significant increases in protein phospeorus ($p<0.01$), and lipid and lipid phosphorus ($p<0.05$) during the preparatory phase when compaced with the postspawning phase (Table 3).

Table 3
Effects of estradiol-17β (E_2) on RNA, DNA, total protein, lipid, protein phosphorus, and lipid phosphorus content in livers of *H. fossilis* during preparatory phase

Batch[a)]	Treatment (daily for 7 days)	A RNA (mg/g wet weight)	B DNA (mg/g wet weight)	C Protein (mg/g wet weight)	D Protein phospho-rus (mg/g wet weight)	E Lipid (mg/g wet weight)	F Lipid phos-phorus (mg/g wet weight)
1- (6)	0.6% saline	5.70 ±0.56	3.20 ±0.08	100.00 ±5.21	10.40[b] ±0.12	72.00[c] ±6.65	16.00[d] ±0.99
2- (5)	E_2 5 μg/fish	8.80** ±0.71	3.20 ±0.10	112.00 ±6.35	15.20** ±0.15	78.00 ±7.63	26.00*** ±1.15
3- (5)	E_2 10 μg/fish	8.10** ±0.85	3.40 ±0.15	120.00* ±5.56	16.40** ±0.31	79.00 ±6.31	26.00*** ±1.16
4- (5)	E_2 50 μg/fish	9.80*** ±0.81	3.30 ±0.09	144.00** ±8.82	19.50*** ±0.86	96.00** ±5.20	28.00*** ±1.15

[a)] No. of fish in each batch is given in parentheses.
Values are given as mean±SEM.
Significance of difference compared between Batch 1 and Batches 2-4 (t test);
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
Significantly different from postspawning phase (two-way ANOVA); [b] $p<0.01$, [c,d] $p<0.05$.
Dose response relationship (two-way ANOVA). Column A, D, E, $p<0.05$; Column C, $p<0.01$; and Column B and F, not significant.

Table 4
Effects of estradiol-17β (E_2) on total protein, protein phosphorus, and lipid phosphorus levels in serum of *H. fossilis* during preparatory phase

Batch[a)]	Treatment (daily for 7 days)	A Serum protein (mg/100 ml)	B Serum protein phosphorus (mg/100 ml)	C Serum lipid phosphorus (mg/100 ml)
1- (6)	0.6% saline (0.2 ml/fish)	6.30[b] ±0.52	14.40[c] ±0.75	6.10[d] ±0.61
2- (5)	E_2 5 μg/fish	12.60* ±0.65	22.20** ±0.93	10.60** ±0.55
3- (5)	E_2 10 μg/fish	12.40* ±0.68	24.90** ±0.99	11.60** ±0.40
4- (5)	E_2 50 μg/fish	17.10* ±0.69	26.20** ±0.98	12.00** ±0.46

[a)] No. of fish in each batch is given in parentheses.
Values are given as mean±SEM.
Significance of difference compared between Batch 1 and Batches 2-4 (*t* test); $*p<0.01$, $**p<0.001$.
Significantly different from postspawning phase (two-way ANOVA); [b,c,d] $p<0.05$.
Dose response relationship (two-way ANOVA). Column B, $p<0.05$; Column A and C, not significant.

Increases in RNA, protein, protein phosphorus, and lipid in the livers in response to various doses of estradiol-17β were found to be dose-dependent. Although treatment with estradiol-17β increased liver lipid phosphorus in both seasons, the magnitudes of elevation were not related to dose (Tables 1 and 3).

All measured biochemical compositions of serum (protein, protein phosporus, and lipid phosphorus) were higher ($p<0.05$) during the preparatory phase than during the postspawning phase (Table 4). Statistically, a dose-response correlation was only established for serum protein phosphorus ($p<0.05$, Tables 2 and 4). Increases in serum protein and serum lipid phosphorus were not dose-dependent. These observations demonstrate that the release of these two vitellogenic constituents is independent of the amount of hormone administered.

DISCUSSION

In this exporiment, preparatory phase fish had higher levels of phosphoprotein and phospholipid in their livers and serum when compared with postspawning phase fish. In hagfish, *Eptatretus stouti*, increased levels of total protein in livers and plasma of vitallogenic females were found when compared with non-vitellogenic females; at the same time, yolk protein was observed only in the plasma of vitellogenic females (Yu *et al.*, 1980).

Estradiol-17β induced the synthesis, as well as the releases of phosphoprotein and phospholipid in *H. fossilis* in both preparatory and postspawning phases of the reproductive cycle. This finding is comparable to those reported for goldfish (De Vlaming *et al.*, 1977a; Hori *et al.*, 1979), winter flounder (Campbell and Idler, 1976), and eel (Olivereau and Olivereau, 1979a, 1979b). De Vlamin *et al.* (1977b) demonstrated that estradiol-17β treatments

increased hepatic lipid in *Notemigonus crysoleucas* during the gonadal preparatory period, but failed to bring about any change during the prespawning season. But during the early spawning season, estrogen elicited increased levels of liver protein and phospholipid in the same fish. Further more, De Vlaming *et al.* (1979) demonstrated that estrogen treatments enhanced levels of organic PO_4, cholesterol, and protein in the serum of *Carassius auratus*. Intramusculear injections of estradiol brought about significant increases in plasma phospholipid, phosphoprotein, calcium, total lipid, and protein in dogfish, *Scyliorhinus canicula* (Crajk, 1978b). Sundararaj and Nath (1981) showed that estrogen induced higher levels of alkali labile phosphoprotein in livers and serum of *H. fossilis*. However, that study ravealed that apart from phosphoprotein, phospholipid is also synthesized in the liver and released into blood under estrogen control. In *H. fossilis*, RNA was synthesized in response to exogenous estrogen administration. Hahn *et al.* (1969) and Callard *et al.* (1972) also showed that estradiol treatments induced liver RNA synthesis in reptiles. Estradiol treatments also elevated levels of RNA, protein, and lipids, as well as ratios of RNA/DNA, protein/DNA, and lipid/DNA in the livers of immature chickens (*Gallus domesticus*) in comparison to controls (Yu and Marquardt, 1973).

Estradiol administration influenced slight variations in response during the two different periods of the reproductive cycle. For example, low doses (5 μg/fish) of estradiol did not significantly change liver protein levels during the postspawning phases whereas similar doses caused elevations in total liver lipid levels, excluding phosphorus-bound lipid. On the other hand, during the preparstory period, low (5 μg/fish) and medium (10 μg/fish) doses of estradiol induced synthesis of phosphorus-bound lipid without affecting total lipid levels in pituitary and ovarian circulating hormones during the post-spawning and preparatory phases of the reproductive cycle. Estrogen has been found to increase parallel to vitellogenin in fish, attaining peak levels at times of maximum gonadal maturation (Crim and Idler, 1978; Bohamon and Lambert, 1981). In catfish, *Clarias batrachus*, progressive elevations in prolactin and gonadotropin levels were observed with the onset of gonadal development in the proparatory phase, but their maximum concentrations were noticed during the spawning phase (Singh and Singh, 1980). Prolactin is also known to alter body and liver lipid levels in several fish species (Lee and Meier, 1967; Meier, 1969; De Vlaming *et al.*, 1975; Pardo and De Vlaming, 1976; Singh, 1981). In the presant study, intact *H. fossilis* were used in both seasons; therefore, prolactin involvement in lipid synthesis cannot be ruled out.

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REFERENCES

Bohemen, Ch. G. Van and J. G. D. Lambert (1981) Estrogen synthesis in relation to estrogen, estradiol and vitellogenin plasma levels during the reproductive cycle of female rainbow trout, *Salmo gairdneri*. *Gen. Comp. Endocrinol.* **45**: 105-114.

Craik, J. C. A. (1978a) Plasma level of vitellogenin in elasmobranch *Scyliorhinus canicula* L. (lesser spotted dogfish). *Comp. Biochem. Physiol.*, **60B**: 9-18.

Craik, J. C. A. (1978b) The effect of oestrogen treatment on certain plasma constituents associated with vitellogenesis in elasmobranch

Scyliorhinus canicula L. *Gen. Comp. Endocrinol.* **35**: 455-464.

Campbell, C.M. and D.R. Idler (1978) Hormonal control of vitellogenesis in hypophysectomized winter flounder (*Pseudopleuronectes americanus* Walbaum). *Gen. Comp. Endocrinol.* **28**: 143-150.

Crim, L.W. and D.R. Idler (1978) Plasma gonadotropin, estradiol, and vitellogenin and gonad phosvitin levels in relation to the seasonal reproductive cycle of female brown trout. *Ann. Biol. Anim. Biochem. Biophys.* **18**: 1019-1025.

Callard, I.P., S.H. Banks and W. Banks (1972) Hepatic protein and nucleic acid content in *Dipsosaurus dorsalis* following hypophysectomy and treatment with estradiol-17β and growth hormone. *Comp. Biochem. Physiol.* **41B**: 503-510.

De Vlaming, V.L., M. Sage and R. Tiegs (1975) A diurnal rhythm of pituitary prolactin activity with diurnal effects of mammalian teleostean prolactin on total body lipid deposition and liver lipid metabolism in teleost fishes. *J. Fish. Biol.* **7**: 717-726.

De Vlaming, V.L., J. Shing, G. Paquette and R. Vuchs (1977a) *In vivo* and *in vitro* effects of estradiol-17β on lipid metabolism in *Notemiqonus crysoleucas*. *J. Fish. Biol.* **10**: 273-285.

De Vlaming, V.L., M.J. Vodicnick, G. Bauer, T. Murphy and D. Evans (1977b) Estradiol-17β effects on lipid and carbohydrate metabolism and on the induction of yolk precursor in goldfish, *Crassius auratus*. *Life Sci.*, **20**: 1945-1952.

De Vlaming, V.L., G. Delabunty, M. Prack and G. Bauer (1979) Estradiol-17β effects on serum organic PO_4, cholesterol and protein in *Carassius auratus*. *Copeia* 1979: 749-751.

Fiske, C.H. and Y. Subba Row (1925) Colorimetric determination of phosphorus. *J. Biol. Chem.* **66**: 375-400.

Folch, J., M. Lees and G.H. Sloane-Stanley (1957) A simple method of isolation and purification of total lipid from animal tissues. *J. Biol. Chem.* **226**: 497-507.

Follet, B.K., T.J. Nicholls and M.R. Redshaw (1968) The vitellogenic response in the south african clawed toad (*Xenopus laevis* Daudin). *J. Cell. Physiol. Suppl.* **72**: 91-102.

Gapp, D.A., S.M. Ho and I.P. Callard (1979) Plasma level of vitellogenin in *Chrysemys* during the annual gonadal cycle. Measurement by specific radioimmunoassay. *Endocrinology* **104**: 784-790.

Hahn, W.E., R.B. Church and A. Gorbman (1969) Synthesis of new RNA species prior to estradiol induced vitellogenesis. *Endocrinology* **84**: 738-745.

Hori, S.H., T. Kodama and K. Tanahashi (1979) Induction of vitellogen in synthesis in goldfish by massive doses of androgens. *Gen. Comp. Endocrinol.* **37**: 306-320.

Lee, R.W. and A.H. Meier (1967) Diurnal variation of the fattening response to prolactin in golden top minnow, *Fundulus chryotus*. *J. Exp. Zool.* **166**: 307-316.

Lowry, O.H., N.J. Rosenbrough, A. Farr and R.J. Randall (1951) Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

Meier, A.H. (1969) Diurnal variations of metabolic responses to prolactin lower vertebrates. *Gen. Comp. Endocrinol. Suppl.* **2**: 55-62.

Munro, H.N. and A. Fleck (1966) *The determination of nucleic acid. In*: Methods of Biochemical Analysis (D. Glick ed.) Interscience Publishers, New York. Vol. 14, 113-176.

Nath, P. and B.I. Sundararaj (1981) Induction of vitellogenesis in the hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). Effects of piscine and mammalian hormones. *Gen. Comp. Endocrinol.* **43**: 191-200.

Nagahama, Y., K. Chan and W.S. Hoar (1976) Histochemistry and ultrastructure of pre- and post-ovulatory follicles in the ovary of goldfish, *Carassius auratus*. *Can. J. Zool.* **54**: 1228-1139.

Olivereau, M. and J. Olivereau (1979a) Estradiol positive feed-back on gonadotropic (GTH) cells in freshwater male silver eels. *Gen. Comp. Endocrinol.* **39**: 247-261.

Olivereau, M. and J. Olivereau (1979b) Effect of estradiol-17β on the cytology of liver, gonads and pituitary and on plasma electrolytes in the female freshwater eel. *Cell Tissue Res.* **199**: 431-454.

Pardo, R.J. and V.L. De Vlaming (1976) *In vivo* and *in vitro* effects of prolactin on lipid metabolism in the cyprinid teleost, *Notemigonus crysoleucas*. *Copeia* 1976: 563-573.

Redshaw, M.R. and B.K. Follet (1971) The crystalline yolk platelet proteins and their soluble plasma precursors in an amphibian *Xenopus leavis*. *Biochem. J.* **124**: 759-766.

Schneider, W.C. (1957) *Determination of nucleic acid in tissue by pentose analysis.* In: Methods in Enzymology (S.P. Colowich and N.O. Kaplan eds.). Academic Press, New York. Vol. 3, 680-684.

Sokol, R. and F.J. Rohlf (1969) *Introduction to Biostatistics.* W.H. Freeman & Co., San Francisco. pp. 186-196.

Singh, A.K. (1979) Effect of thyroxine and thiourea on liver and muscle energy stores in the freshwater catfish, *Heteropneustes fossilis. Ann. Biol. Anim. Biochem. Biophys.* **19**: 1669-1679.

Singh, A.K. (1981) Effect of prolactin on the tissue glycogen and lipid content in the catfish, *Heteropneustes fossilis* (Bloch). *Ind. J. Exp. Biol.* **19**: 425-427.

Singh, A.K. and T.P. Singh (1979) Seasonal fluctuation in lipid and cholesterol content of ovary liver and blood serum in relation to annual sexual cycle in *Heteropneustes fossilis* (Bloch). *Endokrinologie* **73**: 47-54.

Singh, S.P. and T.P. Singh (1980) Prolactin level in relation to gonadotropin concentration during different phases of annual reproductive cycle in freshwater catfish, *Clarias batrachus* (Linn.). *Ann. Endocrinol.* (Paris) **41**: 415-424.

Sundararaj, B.I. and P. Nath (1981) Steroid induced synthesis of vitellogenin in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.* **43**: 201-210.

Upadhyay, S.N., B. Breton and R. Billard (1978) Ultrastrastructure studies on experimentally induced vitellogenesis in juvenile rainbow trout. *Ann. Biol. Anim. Biochem. Biophys.* **18**: 1019-1025.

Wallace, R.A. and D.W. Jared (1969) Studies on amphibian yolk, VIII. The estrogen induced hepatic synthesis of lipophosphoprotein and its selective uptake by the ovary and transformation into yolk platelet proteins in *Xenopus laevis. Develop. Biol.* **19**: 498-526.

Wallace, R.A. and E.W. Bergink (1974) Amphibian vitellogenin properties, hormonal regulation of hepatic synthesis and ovarian uptake and conversion to yolk protein. *Amer. Zool.* **14**: 1159-1175.

Yu, J.Y.-L. and R.R. Marquardt (1973) Effects of estradiol and testosterone on the immature female chicken (*Gallus domesticus*). I. Quantitative changes in nucleic acids, proteins and lipids in the liver. *Comp. Biochem. Physiol.* **46B**: 749-757.

Yu, J.Y.-L., W.W. Dickhoff, Y. Inui and A. Gorbman (1980) Sexual patterns of protein metabolism in liver and plasma of hagfish, *Eptatretus stouti* with special reference to vitellogenesis. *Comp. Biochem. Physiol.* **65B**: 111-117.

Yu, J.Y.-L., W.W. Dickhoff, P. Swanson and A. Gorbman (1981) Vitellogenesis and its hormonal regulation the in Pacific hagfish, *Eptatretus stouti* L. *Gen. Comp. Endocrinol.* **43**: 492-502.

Yaroz, Z. and L. Widzer (1978) The control of vitellogenesis by ovarian hormones in the lizard *Xantusia vigilis. Comp. Biochem. Physiol.* **69**: 279-284.

Yaron Z., A. Terkatin-Shimony, Y. Shaham and H. Salzer (1977) Occurrence and biological activity of estradiol-17β in the intact and ovariectomized *Tilapia aurea* (Chichlidae: Teleostei) *Gen. Comp. Endocrinol.* **33**: 45-52.

淡水性雌鯰魚 *Heteropneustes fossilis* 在年生殖週期兩個不同期間受雌性素刺激引起之磷蛋白與磷脂之合成與釋放

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本文的目的在研究在排卵後期與排卵前期之年生殖週期時，對 *Heteropneustes fossilis* 施於不同劑量之動情素，所引起之肝與血中磷蛋白與磷脂之變化。在排卵後期，三種動情素劑量（每條魚5微克、10微克、或50微克，共7天）。都增加肝中 RNA 含量，而不改變 DNA 含量。在排卵前期，三種不同劑量之動情素都增加肝與血中之磷蛋白與磷脂；而在排卵後期，只有中劑量（每條魚10微克）才增加肝與血中之磷蛋白與磷脂濃度。而肝之蛋白質濃度，在三種不同劑量之動情素刺激下都增加。動情素對磷脂合成之影響在不同生殖週期而略有不同：在排卵後期，低劑量動情素引起肝之脂質合成，而不影響磷酯合成但在排卵前期，中劑量與高劑量動情素都促進磷脂合成，但不影響肝之脂質合成。