Ann. Biol. anim. Bioch. Biophys., 1978, **18** (4), 949-956.

Circadian responses of teleostean oocytes to gonadotropins and prostaglandins determined by cyclic AMP concentration

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Summary. The work describes the circadian rhythm of teleostean oocyte responses to exogenous gonadotropins and prostaglandins as determined by the level of cyclic AMP accumulation, and the influence of photoperiodicity.

Mature females, *Mugil cephalus*, were divided into two groups and acclimated under a constant photoperiod regime of equal phases (12 L/12 D) at 21 $\pm$ 1 °C for 45 days. Two photoperiod conditions were used. One timed the onset of the light phase at 6 a. m. and the other at 6 p. m. The oocyte responses to NIH-LH, NIH-FSH, SG-G 100, prostaglandins E_2 and F_{2a} were examined at 3-hr intervals. The concentrations of cyclic AMP, assayed by the protein binding technique, were used to indicate the sensitivity of the oocytes to the hormones.

The circadian rhythm of oocyte response to gonadotropins and prostaglandin E_2 appeared to be « timed » by the onset of the light phase. Distinct increases of cyclic AMP accumulation generally occurred at 3 and 12 hr after exposure to light. In the dark phase, synchronized increases were not observed. The circadian rhythm of the endogenous cyclic AMP level was suggested as a reflection of a temporal relationship between the oocyte response and gonadotropin-surge in the plasma. An additive effect of LH and SG-G 100 in activating adenyl cyclase system was further indicated.

Introduction.

A diurnal rhythm of pituitary gonadotropin synthesis and release has been demonstrated in the teleosts *Salvelinus fontinalis*, *Salmo gairdneri* and *Notemigonus crysoleucas* (O'Connor, 1972 ; De Vlaming and Vodicnik, 1977). The physiological significance of this diurnal rhythm is not understood. However, temporal variation in gonadal response to gonadotropins, related to the diurnal cycle of pituitary gonadotropin content, was demonstrated in *N. crysoleucas*, and a maximal stimulatory effect of gonadotropins for inducing ovulation *in vitro* was reported in the late period of light phase in *Oryzias latipes* (Hirose and Donaldson, 1972 ; Hirose and Hirose, 1972).

Interaction of gonadotropins at the ovarian level with a highly specific membrane-bound receptor which triggers enzymatic production of cyclic AMP has been established by Ahren *et al.* (1969), Mason *et al.* (1973) and others. Both carp and salmon gonadotropins have been shown to activate the adenyl cyclase system in goldfish

and salmon (Fontaine *et al.*, 1970 ; Menon and Smith, 1971 ; Fontaine *et al.*, 1972). Prostaglandins, particularly those of the E series, have been shown to mimic the stimulatory action of LH *in vitro* on the formation of cyclic AMP by intact ovaries of the mouse and rat (Kuehl *et al.*, 1970 ; Lamprecht *et al.*, 1973). The cyclic AMP then acts as an intracellular second messenger and mediates biological actions common to LH and PGE_2 on the ovaries. These include the induction of ovum maturation (Tsafriri *et al.*, 1972*a*, *b*), luteinization (Channing, 1970 ; Kolena and Channing, 1971), ovarian steroidogenesis (Pharriss *et al.*, 1968 ; Speroff and Ramwell, 1970 ; and others), and ovarian protein kinase activity (Lamprecht *et al.*, 1973).

Biological rhythms are either endogenous to the organisms or driven by environmental cues. Synchronization depends upon external entraining agents. Light and temperature have been considered two critical variables. The stimulatory effects of different amounts of different hormones could therefore be determined by the temporal relationship between hormones, receptors, and cyclic AMP production.

This report describes the circadian rhythm of oocyte responses to exogenous gonadotropins and prostaglandins *in vitro*, as determined by the level of cyclic AMP accumulation, and the influence of photoperiodicity.

Materials and Methods.

Six mature female mullet, *Mugil cephalus* L., with oocytes at the tertiary yolk globule stage and larger than 500 μ, were divided into two groups and maintained in a constant photoperiod regime of equal phases (12L/12D) at 21 $\pm$ 1 °C for a period of 45 days. Two photoperiod conditions were used. One timed the onset of the light phase at 6 a.m., and therefore simulated natural conditions (normal light cycle) ; the other timed the onset of the light phase at 6 p.m., and therefore reversed the conditions (reversed light cycle). At the end of the acclimation period, the oocytes developed beyond a mean diameter of 600 μ, at which stage they are known to be effectively responsive to SG-G100 (Kuo *et al.*, 1974). Samples of oocytes were withdrawn through a polyethylene cannula from every fish at 3-hr time intervals throughout a 24-hr period.

The samples of oocytes were preincubated for 30 min in Tris buffer solution (pH 7.4), and then incubated in duplicate subsamples in Tris buffer containing theophylline and 2-Mercaptoethanol together with one of the following hormones :

(i) NIH-LH-B10, sp. potency 1.06 $\times$ NIH-LH-S1 : 5 μg/ml ;
(ii) NIH-FSH-B1, sp. potency 0.49 $\times$ NIH-FSH-S1 : 5 μg/ml ;
(iii) SG-G100, sp. potency 0.11 $\times$ NIH-LH-S1 : 5 μg/ml ;
(iv) Prostaglandin E_2(PGE_2) : 0.5 μg/ml ;
(v) Prostaglandin F_{2a}(PGF_{2a}) : 0.5 μg/ml.

At the end of the 5-min incubation period, the subsamples of oocytes were homogenized in 6 p. 100 Trichloroacetic acid solution. The proteins were quantified by the method described by Lowry *et al.* (1951). The levels of cyclic AMP in each sample were measured by the protein binding assay method described by Wombacher and Korber (1971) and Tsang *et al.* (1972).

Results.

The diurnal variations in the endogenous cyclic AMP levels in the mature oocytes are illustrated in figure 1. Levels of cyclic AMP varied between 1.89 and 2.65 pMole/mg protein for those fish exposed to the normal light phase, and between 2.34 and 2.69 pMole/mg protein for those in reversed conditions. Although variations in the levels of endogenous cyclic AMP concentration were recorded, they were not different statistically at 5 p. 100 significant level by student-t test and analysis of variance throughout the 24-hr cycle. There were also no differences statistically in the endogenous cyclic AMP levels between fish exposed to the two different light conditions.

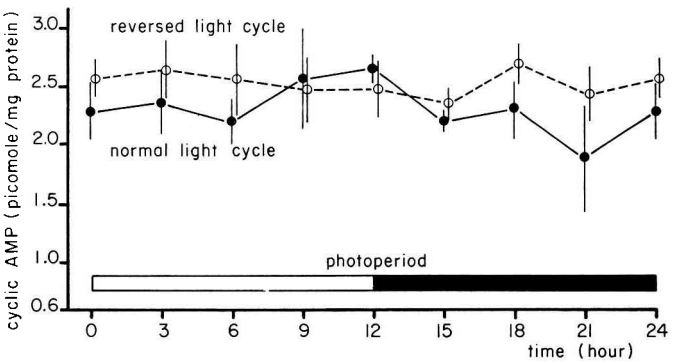

FIG. 1. — *Endogenous cyclic AMP levels in the mature oocytes of* Mugil cephalus (mean pMole/mg protein ± S. E. M.). (———) females exposed to normal light cycle, and (- - - -) for those exposed to reversed light cycle. The time represents hours after the onset of the light phase.

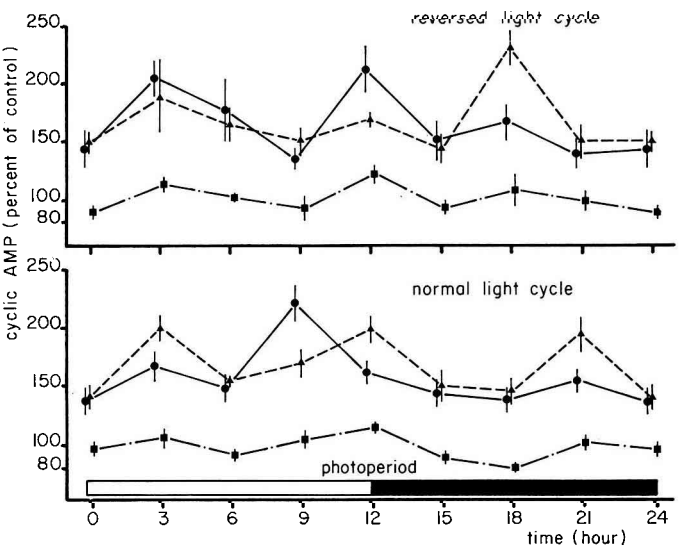

FIG. 2. — *Diurnal rhythm of cyclic AMP accumulation* (mean ± S. E. M.) *in mature oocytes exposed to LH* (———), *FSH* (— · —), *and SG-G* 100 (- - -). The time represents hours after the onset of the light phase.

The diurnal rhythms of mature oocyte response to each of the three gonadotropins was similar in form (fig. 2). In the light phase of both normal and reversed light conditions, distinct increases of cyclic AMP accumulation resulted from gonadotropin stimulation *in vitro*. This generally occurred at 3 and 12 hr after exposure to light. Both LH and SG-G100 appeared to be equally effective in stimulating cyclic AMP synthesis. In the dark phase, synchronized increases were not observed. Response to the gonadotropins increased 9 hr after the onset of darkness in normal conditions, and 6 hr after darkness in the reversed conditions. It also appeared that SG-G100 was more effective than LH.

The gonadotropin FSH was much less effective than either LH or SG-G100 in activating the adenyl cyclase system. The levels of cyclic AMP accumulation under FSH stimulation varied between 82-115 p. 100 of the endogenous level in normal conditions, and between 90-122 p. 100 in the reversed conditions.

Response of the oocytes to the two prostaglandins varied. The rhythmic response to PGE_2 was similar to that effected by the LH stimulation for both phases and light conditions (fig. 3). The effects of PGF_{2a} were greatest at 9 and 12 hr after exposure to light in normal conditions, and 6 hr before and after darkness in the reversed conditions. Although PGF_{2a} was less effective in stimulating cyclic AMP synthesis in the oocytes, the response of the oocytes to it was similar to that stimulated by LH and PGE_2 in the light phase under normal conditions. However, the rhythmic trend was directly opposite from these two hormones under reversed conditions.

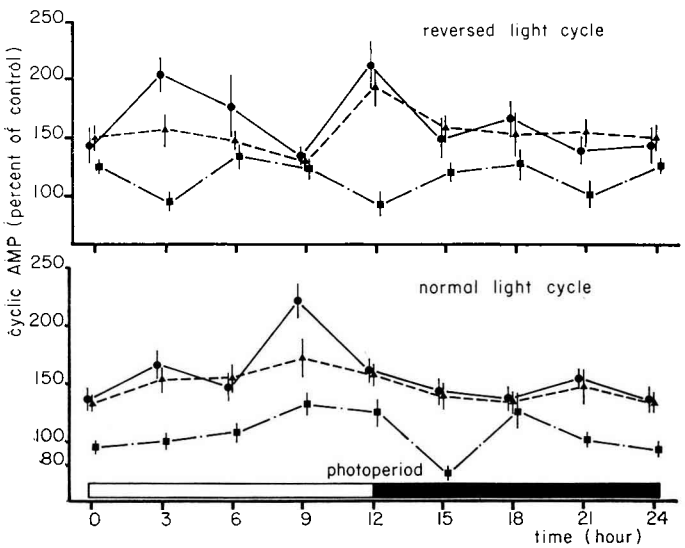

FIG. 3. — *Diurnal rhythm of cyclic AMP accumulation* (mean ± S. E. M.) *in mature oocytes exposed to LH* (———), PGE_2 (- - -), PGF_2 (—·—·). The time represents hours after the onset of the light phase.

The diurnal rhythm of the cyclic AMP levels in oocytes exposed to PGF_{2a} are illustrated in figure 4. There is the suggestion that the diurnal variation of oocyte response to PGF_{2a} is not related to this particular photoperiod regime, but might reflect

either an endogenous physiological rhythm or another unknown environmental influence.

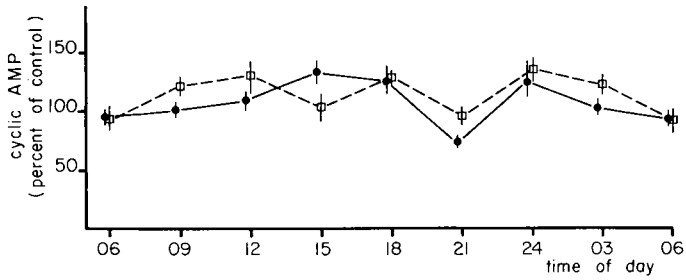

FIG. 4. — *levels of cyclic AMP accumulation in the mature oocytes under prostaglandin* $F_{2\alpha}$ *stimulation.* (———) represents the females exposed to normal light cycle and (- - -) represents those exposed to reversed light cycle.

Discussion.

Evidence by several workers (Koch *et al.*, 1974) suggests that the effect of hormones on different tissues through the activation of the adenyl cyclase system is determined largely by the capacity of the tissues to respond and the amount of hormone present. Continuous presence of the hormone at the binding site of the plasma membrane is necessary to activate the system, but only fractional presence at the receptor site is necessary to induce maximum production of cyclic AMP by the target tissues. In addition, some of the biological effects of LH and HCG on ovarian tissues are reported to require only brief exposure to the hormone (Perklev *et al.*, 1971 ; Lindner *et al.*, 1973), which then triggers an irreversible biochemical reaction.

The maximum accumulation of cyclic AMP in the mature oocytes of grey mullet was recorded for total levels of 15 μg LH or SG-G100. This level was comparable to the pituitary contents reported in *N. crysoleucas*, which ranged between 6.1-34.0 μg of SG-G100 during the 24-hr cycle (de Vlaming and Vodicnik, 1977).

In the experiments, responses to LH and SG-G100 were more pronounced than that of FSH at concentrations of 5 μg/ml. Similar results were reported in rat ovaries (Mason *et al.*, 1973), although Marsh *et al.* (1972) showed that stimulation of cyclic AMP synthesis in rabbit Graafian follicles by FSH was due to LH contamination. Several workers have indicated that LH or HCG, and SG-G100, are effective in inducing ovulation in teleosts, but not FSH (reviewed by de Vlaming, 1974). The types of pituitary gonadotropin cells present in the teleostean fishes are still the subject of conflicting arguments. Results with FSH in this study indicated that there was little response to this gonadotropin as shown by the cyclic AMP accumulation in the 24-hr cycle.

SG-G100 and LH, at a concentration of 5 μg/ml, are equally effective in stimulating adenyl cyclase activity in the mature oocytes of mullet, although the biological potency of SG-G100 is about one-tenth that of LH preparation as indicated by the chick testis radiophosphate uptake assay. Because of the specificity and lack of physiological cross-reaction of pituitary gonadotropins between heterologous species of vertebrates, the assayed potency of the hormones from heterologous species is not comparable if

the assay system of phylogenetically different species is used. Evidence suggests that mammalian gonadotropins are active in nonmammalian species, whereas pituitaries of nonmammalian species, particularly poikilotherms, tend to be devoid of activity when tested in mammals (Channing *et al.*, 1974). Scanes *et al.* (1972) reported a poor cross-reaction of poikilotherm gonadotropins in the chicken LH radioimmunoassay system. In contrast, piscine pituitary gonadotropins were shown to be effective in teleost systems, although they showed a specificity among teleosts (Breton *et al.*, 1973). Species specificity within teleosts was reported to be minor compared with that between teleost and mammalian species (Fontaine *et al.*, 1972). The present study was not intended to compare the effectiveness of gonadotropin preparations from the different sources, but to determine any oocyte response to these hormones throughout the 24-hr cycle.

Experimental results suggested that the stimulatory action of PGE_2 on cyclic AMP synthesis was more potent than that of PGF_{2a} on mature oocytes of the mullet. In general, PGE_2 has been found to be more potent than PGF_{2a} in promoting progesterone synthesis *in vitro* (Speroff and Ramwell, 1970), but less potent in the luteolysis of rat corpora lutea (Labhsetwar, 1975). Okamura *et al.* (1972), Jalabert and Szöllösi (1975), and Jalabert (1976) indicated that PGF_{2a} may play an important role in follicle rupture (ovulation) through the stimulation of smooth muscle fibers present in the ovarian stroma as well as in the follicle walls. Kuehl (1974) further suggested that PGF_{2a} was more selective in stimulating cyclic GMP, and that its role in the production of cyclic AMP was minor.

In the 24-hr cycle, marked increases in cyclic AMP accumulation in the oocytes were recorded at certain times after exposure to the light phase, regardless of the time of day. The circadian rhythm of oocyte response to gonadotropins and PGE_2 appeared to be « timed » by the onset of the light phase. The photosensivity rhythm of organisms determines whether light will stimulate the hypothalamo-hypophyseal gonadal axis (Stetson *et al.*, 1975) or not. The circadian rhythm of the endogenous cyclic AMP level might be reflecting a temporal relationship between the oocyte response and the pituitary gonadotropin level or gonadotropin-surge in the plasma. De Vlaming and Vodicnik (1977) reported that the gonadal response to gonadotropin treatment was maximal when the pituitary gonadotropin level was minimal for *N. crysoleucas* maintained in a 15 1/2 L/8 1/2 D photoperiod regime at 15 °C, Gonadal responses might therefore be related to the gonadotropin-surge in the plasma, and the hormones act as triggers iniating biochemical reactions that persist after the hormones are no longer detectable. In the absence of data on diurnal rhythms in pituitary gonadotropin level or gonadotropin-surge in the grey mullet, this relationship has not yet been determined. The increase of cyclic AMP accumulation in the oocytes responding to exogenous LH or SG-G100 *in vitro*, was observed in most cases, if not all, when the endogenous cyclic AMP levels were relatively high. The results further indicate the additive effect of these gonadotropins in activating adenyl cyclase system.

Symposium sur la Reproduction des Poissons
Paimpont, France, 19-21 *septembre* 1977.

Acknowledgments. — We are grateful to the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH for providing the NIH-LH and NIH-FSH, and to Drs. J. W. Lauderdale and J. E. Pike of the Upjohn Co. for Prostaglandins, used in this study. We thank Dr. Colin E. Nash for editing the manuscript, and Ms. Dottie Rosinsky for preparation of illustrations. This work was supported by the US Agency for International Development, Grant No. AID/ta-C-1189.

Résumé. Ce travail décrit le rythme circadien de la réponse des ovocytes d'un poisson téléostéen aux gonadotropines exogènes et aux prostaglandines.

Des femelles matures, *Mugil cephalus*, sont divisées en deux groupes et acclimatées pendant 45 jours à une photopériode constante 12 L-12 N à 21 $\pm$ 1 °C. Le début de la période claire est à 6 h pour un groupe et 18 h pour l'autre. La réponse des ovocytes à NIH-LH, NIH-FSH, SG-G 100, et aux prostaglandines E_2 et $F_{2\alpha}$ est examinée toutes les 3 h. La sensibilité des ovocytes aux hormones est testée par le dosage de l'AMP cyclique.

Le rythme circadien de la réponse des ovocytes aux gonadotropines et à la prostaglandine E est déterminé par le début de la période claire. Une augmentation notable de l'accumulation d'AMPc se produit généralement à 3 et 12 h après l'allumage. Pendant la période sombre on n'observe pas d'augmentations synchronisées. Le rythme circadien du niveau d'AMPc endogène apparaît comme un reflet de la relation temporelle entre la réponse de l'ovocyte et la décharge de gonadotropine dans le plasma. Il semble exister un effet additif de LH et SG-G 100 pour activer le système adényl cyclase.

References

AHRÉN K., HAMBERGER L., RUBINSTEIN L., 1969. Acute *in vivo* and *in vitro* effects of gonadotropins on the metabolism of the rat ovary, 327-354. In McKERNS K.W., *The gonads*. Acad. Press, New York.

BRETON B., BILLARD R., JALABERT B., 1973. Spécificité d'action et relations immunologiques des hormones gonadotropes de quelques téléostéens. *Ann. Biol. anim. Bioch. Biophys.*, **13,** 347-362

CHANNING C. P., 1970. Influence of the *in vivo* and *in vitro* hormonal environment upon luteinization of granulosa cells in tissue culture. *Recent Progr. Hormone Res.*, **26,** 589-622.

CHANNING C. P., LICHT P., PAPKOFF H., DONALDSON E. M., 1974. Comparative activities of mammalian, reptilian, and piscine gonadotropins in monkey granulosa cell cultures. *Gen. comp. Endocr.*, **22,** 137-145.

DE VLAMING V. L., 1974. Environmental and endocrine control of teleost reproduction, 13-83. In SCHRECK C. B., *Control of sex in fishes*. Virginia Polytech. Inst. State Univ., Blacksburg, U. S. A.

DE VLAMING V. L., VODICNIK M. J., 1977. Diurnal variations in pituitary gonadotropin content and in gonadal response to exogenous gonadotropin and prolactin in *Notemigonus crysoleucas*. *J. Fish Biol.*, **10,** 371-384.

FONTAINE Y. A., BURZAWA-GÉRARD E., DELERUE-LEBELL N., 1970. Stimulation hormonale de l'activité adénylcyclasique de l'ovaire d'un poisson téléostéen le Cyprin, *Carassius auratus* L. *C. R. Acad. Sci. Paris, Série D,* **271,** 780-783.

FONTAINE Y. A., SALMON C., FONTAINE-BERTRAND E., BURZAWA-GÉRARD E., DONALDSON E. M., 1972. Comparison of the activities of two purified fish gonadotropins on adenyl clase activity in the goldfish ovary. *Can. J. Zool.*, **50,** 1673-1676.

HIROSE K., HIROSE H., 1972. Biological study on ovulation *in vitro* of fish. II. Differences of ovulation rates in *Oryzias latipes* at various starting hours of incubation. *Bull. jap. Soc. Sci. Fish.*, **38,** 33-42.

HIROSE K., DONALDSON E. M., 1972. Biological study on ovulation *in vitro* of fish. III. The induction of *in vitro* ovulation of *Oryzias latipes* oocytes using salmon pituitary gonadotropin. *Bull. jap. Soc. Sci. Fish.*, **38,** 97-100.

JALABERT B., 1976. *In vitro* oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*), and goldfish (*Carassius auratus*). *J. Fish. Res. Bd. Canada*, **33,** 974-988.

JALABERT B., SZÖLLÖSI D., 1975. *In vitro* ovulation of trout oocytes : effect of prostaglandins on smooth muscle-like cells of the theca. *Prostaglandins*, **9**, 765-778.

KOCH Y., ZOR U., CHOBSIENG P., LAMPRECHT, S. A., POMERANTS S., LINDNER H. R., 1974. Binding of luteinizing hormone and human chorionic gonadotropin to ovarian cells and activation of adenylate cyclase. *J. Endocr.*, **61**, 179-191.

KOLENA J., CHANNING C. P., 1971. Stimulatory effects of gonadotropins on the formation of cyclic adenosine 3',5'-monophosphate by porcine granulosa cells. *Biochim. biophys. Acta*, **252**, 601-606.

KUEHL F. A. Jr., 1974. Prostaglandins, cyclic nucleotides and cell function. *Prostaglandins*, **5**, 325-340.

HUEHL F. A. Jr., HUMES J., TARNOFF J., CIRILLO V., HAMM E. A., 1970. Prostaglandin receptor site : Evidence for an essential role in the action of luteinizing hormone. *Science*, **169**, 883-886.

KUO C. M., NASH C. E., SHEHADEH Z. H., 1974. A procedural guide to induce spawning in grey mullet (*Mugil cephalus* L.). *Aquaculture*, **3**, 1-14.

LABHSETWAR A. P., 1975. Prostaglandins and studies related to reproduction in laboratory animals, 242-270. In KARIM S. M. M., *Prostaglandins and reproduction*. Univ. Park Press, Baltimore, U. S. A.

LAMPRECHT S. A., ZOR U., TSAFIRI A., LINDNER H. R., 1973. Action of Prostaglandin E_2 and of luteinizing hormone on ovarian adenylate cyclase, protein kinase and ornithine decarboxylase activity during postnatal development and maturity in the rat. *J. Endocr.*, **57**, 217-223.

LINDNER H. R., TSAFRIRI A., LIEBERMAN M. E., ZOR U., KOCH Y., BAUMINGER S., BARNEA A., 1973. Gonadotropin action on cultured Graafian follicles : induction of maturation division of the mammalian oocyte differentiation of the luteal cell. *Recent Progr. Hormone. Res.*, **30**, 79-138.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L., RANDALL R. J., 1951. Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.

MARSH J. M., MILLS T. M., LEMAIRE W. J., 1972. Cyclic AMP synthesis in rabbit Graafian follicles and the effect of luteinizing hormone. *Biochim. biophys. Acta*, **273**, 389-394.

MASON N. R., SCHAFFER R. J., TOOMEY R. E., 1973. Stimulation of cyclic AMP accumulation in rat ovaries *in vitro*. *Endocrinology*, **93**, 34-41.

MENON K. M. J., SMITH M., 1971. Characterization of adenyl cyclase from the testes of chinook salmon. *Biochemistry*, **10**, 1186-1190.

O'CONNOR J. M., 1972. Pituitary gonadotropin patterns in pre-spawning brook trout. *Salvelinus fontinalis*, rainbow trout, *Salmo gairdneri* and *leopard* frogs, *Rana pipiens*. *Comp. Biochem. Physiol.*, **43A**, 739-746.

OKAMURA H., VIRUTAMASEN P., WRIGHT K., WALLACH E., 1972. Ovarian smooth muscle in the human being, rabbit and cat. *Amer. J. Obstet. Gynecol.*, **112**, 183-191.

PERKLEV T., BERGLUND H. B., AHRÉN K., 1971. Effect of HCG, LH, and anti-HCG serum on the lactic acid production of the prepubertal rat ovary. *Acta endocr., Kbh.*, **68**, 31-40.

PHARRISS B. B., WYNGARDEN L. J., GUTKNECHT G. D., 1968. Biological interactions between prostaglandins and luteotrophins in the rat, 121 $_x$-129. In ROSENBERG E., *Gonadotrophins*. Geron-X Inc., Palo Alto, California, U. S. A.,

SCANES C. G., FOLLETT B. K., GOOS H. J. T., 1972. Cross-reation in a chicken LH radioimmunoassay with plasma and pituitary extracts from various species. *Gen. comp. Endocr.*, **19**, 596-600.

SPEROFF L., RAMWELL P. W., 1970. Prostaglandin stimulation of *in vitro* progesterone synthesis. *J. clin. Endocrinol. Metab.*, **30**, 345-350.

STETSON M. H., ELLIOTT J. A., MENAKER M., 1975. Photoperiodic regulation of hamster testis : circadian sensitivity to the effects of light. *Biol. Reprod.*, **13**, 329-339.

TSAFRIRI A., LINDNER H. R., ZOR U., LAMPRECHT S. A., 1972*a*. *In vitro* introduction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. *J. Reprod. Fert.*, **31**, 39-50.

TSAFRIRI A., LINDNER H. R., ZOR U., LAMPRECHT S. A., 1972*b*. Physiological role of prostaglandins on the induction of ovulation. *Prostaglandins*, **2**, 1-10.

TSANG C. P.W., LEHOTAY D. L., MURPHY B. E. P., 1972. Competitive binding assay for adenosine 3':5'-monophosphate implying bovine adrenal protein. *J. clin. Endocrinol. Metab.*, **35**, 809-817.

WOMBACHER H., KORBER F., 1971. A simple competitive protein binding assay for cyclic adenosine monophosphate. *Z. klin. Chem. klin. Biochem.*, **10**, 260-266.