electric pan balance. Food and water were given ad libitum. The first experimental photophase was always in the phase with the pretreatment, and commenced at 06.00 h. Data were analyzed by Student's t-test; each group was compared with every other group.

Results and discussion. The data summarized in the figure demonstrate that unlike the situation in Japanese quails[13,14] increase in number of light cycles or presence of more light during the PSP is not associated with synthesis and release of additional GTH (gonadotropic hormone) in the rosefinches. Since rosefinches have PSP of about 5 h (12/13 to 17/18 h after dawn[8]), obviously in all the experiments, except 12L:12D, only 1 of the light pulses occur during the PSP (fig.). 12L:12D has thus no coincidence of light with PSP and hence no testicular response[12]. Further, the larger testes in the 18L:6D birds does not appear to be due to presence of more light during the PSP, since the 6L:6D group had an equal amount of illumination during the PSP but did not have bigger gonads than other stimulated groups. Moreover, the average CTW for birds in 18L:6D is not significantly greater than for birds in 3L:3D, 4L:4D or 6L:6D.

The results are comparable to our earlier studies on this species[7,8] and others[3], and are consistent with the hypothesis[15] that the daily photoperiod has a dual role: 1. as entrainer of the circadian rhythmicity in 'photosensitivity', and 2. as an inducer, if it is long enough to extend into the PSP of the entrained circadian rhythmicity in 'photosensitivity'. Thus, on the basis of our this and earlier studies[7,8] we believe that the photoperiodic gonadal responses in *C. erythrinus* are regulated by circadian rhythms, and that the mechanism involved during time-measurement is easily explainable within the framework of the 'external-coincidence' model.

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Copurification of prostaglandin $F_{2\alpha}$ receptors with rat uterine plasma membranes[1]

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Summary. Myometrial homogenates of estrogen-treated nonpregnant rats were fractionated by differential and discontinuous sucrose gradient centrifugation. Binding of $PGF_{2\alpha}$ was maximal in membrane fraction which showed the highest specific activity of 5'-nucleotidase, a marker for plasma membrane.

Although the uterotonic action of $PGF_{2\alpha}$ has been extensively documented and its functional role in parturition often implied[3,4], the intimate mechanism of action of PG-induced uterine contractions is still poorly understood. However, specific uptake of $PGF_{2\alpha}$ by uterine preparations suggests that PGs trigger contractile activity by first binding with high affinity to discrete sites on myometrial cells. Such interaction may alter certain cell functions (e.g. calcium transport) leading to muscular contractions. It is not clear however, whether PGs bind to the exterior of cell membranes in a fashion that is analogous to peptide hormones, or act in the cell interior where they interact with cellular components (e.g. sarcoplasmic reticulum). In this study we attempted to elucidate this question by correlating the increase in the specific activity of a typical marker enzyme of plasma membrane with that of the binding of $PGF_{2\alpha}$ to the same rat uterine preparations.

Materials and methods. Virgin Sprague Dawly rats received daily doses of diethylstilbestrol (1 mg i.p.) for 3 consecutive days. 24 h after the last injection the rats (12/experiment) were killed and the uterine horns were removed and the pooled myometrial tissues were first minced with scissors and then homogenized in ice-cold buffer (0.25 M sucrose, 10 mM Tris-HCl, pH 7,5, 1 mM mercaptoethanol and 1 mM Ca Cl_2), using a Polytron homogenizer at rheostat setting 5 for 3×15 sec. The homogenate was filtered through 2 layers of surgical gauze and the filtrate centrifuged at $600\times g$ for 15 min. The pellet was resuspended in buffer and stored at -20 °C (fraction F_1). The supernatant was centrifuged at $2000\times g$ for 20 min. The pellet was

Distribution of marker enzymes in rat myometrial fractions obtained by differential centrifugation

	$Mg^{2+}(Na^+ + K^+)$-ATPase	Mg^{2+}-ATPase	5'-Nucleotidase
Homogenate	20.5 ± 2.7	29.0 ± 7.2	10.3 ± 1.3
F_1 fraction	30.4 ± 3.9	32.5 ± 7.0	8.2 ± 2.6
F_2 fraction	45.2 ± 12.2	32.0 ± 4.9	9.2 ± 1.6
F_3 fraction	52.6 ± 9.2	53.3 ± 14.1	17.2 ± 0.2
F_4 fraction	102.8 ± 21.4	88.3 ± 27.8	26.5 ± 2.4

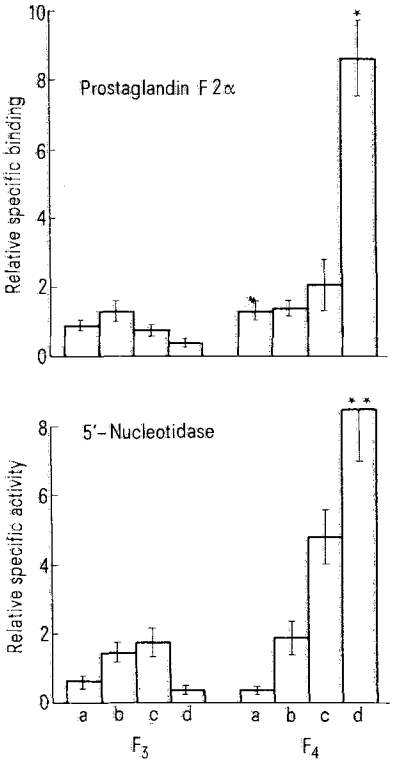
Enzyme activity is expressed as μmoles P/mg protein/h. Mean values were calculated from data obtained with 3 different preparations ± SEM.

briefly homogenized in 40 ml buffer and recentrifuged at 2000 × g for 20 min. The pellet (fraction F_2) was resuspended and frozen. The combined supernatant was centrifuged at 10,000 × g for 15 min. The pellet was resuspended and designated F_3. The supernatant was centrifuged at 60,000 × g for 60 min and is referred to as F_4. (The supernatant of this fraction contained only 5–15% enzyme activity/mg protein in comparison to the homogenate and was discarded). $F_3$and F_4 were further fractionated by discontinuous sucrose gradient centrifugation[5]. Four layers obtained at sucrose interfaces of 10–30%, 30–35%, 35–45% and 45–65% were disignated a, b, c and d respectively and were used for enzyme assay. For binding studies the subfractions were washed with buffer and centrifuged at 90,000 × g for 60 min.

The acitivity of 5′-nucleotidase, $Mg^{2+}-(Na^+ + K^+)$ATPase and Mg^{2+}-dependent ATPase was determined as described previously[6]. Protein determination was carried out by the method of Lowry et al[7].

$PGF_{2\alpha}$ binding was assayed by incubating [^{3}H]$PGF_{2\alpha}$ (sp. act. 150–180 Ci/mmol, New England Nuclear, Boston, MA) alone at a concentration of 10^{-7} M and in the presence of 5×10^{-5} M unlabeled PGF_2 (Enzaprost F, Chinoin, Budapest) with membrane preparations (about 0.2 mg protein) at 20 °C for 60 min in a final volume of 0.2 ml and under continuous shaking. Bound $PGF_{2\alpha}$ was separated from free by filtration through Sephadex G-50 columns and the radioactivity measured in 1-ml fractions collected directly into scintillation vials.

Results and discussion. The distribution of enzyme acitivities of 3 plasma membrane markers in the myometrial fractions obtained by differential centrifugation show a 2.5–5-fold enrichment in the F_4 (10,000–60,000 g pellet) with some increase in the F_3 (2000–10,000 g pellet) (table). For this reason only F_3 and F_4 were further fractionated by gradient centrifugation. Specific binding of $PGF_{2\alpha}$ and the acitivity of 5′-nucleotidase, the enzyme chosen as the plasma membrane marker, are illustrated in the figure. It is evident that $PGF_{2\alpha}$ binding was the highest in the same subfraction which showed the greatest enrichment of the marker enzyme. Such preferential uptake of $PGF_{2\alpha}$ by preparation of avian uterus enriched in sarcolemma has recently been described[6]. Similarly, Crankshaw et al.[8] have reported the selective binding of PGE_1 to the plasma membrane enriched fraction of nonpregnant human myometrium. Taken together, these studies lend support to the notion that $PGF_{2\alpha}$ binds selectively to the cell membrane as the 1st step in its uterotonic action. Because calcium channel blockers or calcium deficient medium inhibit the contractile effect of $PGF_{2\alpha}$ in isolated mammalian[9] and avian[10] uterine strips it seems safe to assume that such binding is associated with an increased calcium influx. However, in bovine myometrial preparations PGE_2 binds to both sacrolemma and sarcoplasmic reticulum with similar affinity and an intracellular site of action by this prostaglandin on calcium transport has been proposed[11]. Because sarcoplasmic membranes probably copurify to some extent with sarcolemmal membranes in the present system, our data do not exclude such a mechanism of action. However, the fact that $PGF_{2\alpha}$ binding was closely associated with the cellular fraction richest in plasma membrane provides supportive evidence that such binding represents the 1st step in the action of $PGF_{2\alpha}$ in the uterine smooth muscle.



Localization of $PGF_{2\alpha}$ binding and 5′-nucleotidase activity in rat myometrial membrane fractions. F_3 and F_4 fractions were obtained by differential centrifugation and subfractions a, b, c and d after centrifugation through discontinuous sucrose gradient. The specific activity of the enzyme is expressed relative to the average activity of the subfractions of F_3 (mean of 2 experiment ± SD). Specific binding is illustrated relative to the average binding (cpm/mg protein) of the 4 subfractions in F_3 and represents the mean of 3 experiments ± SE. *Significantly different ($p < 0.01$) from all specific binding values; **Significantly different ($p < 0.05$) from all except F_4/c enzyme activity.

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