In conclusion, the present study directly demonstrates the capacity of both full-term and early human placentas for the synthesis of placenta-specific SP_1 glycoprotein.

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Day and night levels of hormones in male rhesus monkeys kept under controlled or constant environmental light[1]

A.K. Dubey, C.P. Puri, V. Puri and T.C. Anand Kumar[2]

Experimental Biology Unit, WHO Collaborating Centre for Research and Training in Human Reproduction, All India Institute of Medical Sciences, New Delhi-110029 (India), August 24, 1981

Summary. The marked differences between day and night levels of testosterone, cortisol, prolactin and bioactive luteinizing hormone observed in adult male rhesus monkeys maintained at 12 h light:12 h dark schedule are not changed by exposing monkeys to constant illumination for a period of up to 15 days. These findings suggest that the photoperiodicity of environmental light may not be the principal determinant for the occurrence of the diurnal rhythms observed in the circulating levels of the 4 hormones studied.

The photoperiodicity of environmental light is known to bear a causal relationship with the timing of many reproductive events, including the circadian rhythmicity of the circulating levels of hormones in most of the lower mammals so far studied. This relationship makes it possible to predict occurrence of certain reproductive events in animals maintained under well controlled light:dark schedules. This is particularly true for the albino rat which is amongst the most commonly used experimental animal.

Rhesus monkeys *(Macaca mulatta)* maintained under controlled environmental lighting conditions also exhibit distinct diurnal rhythms in their circulating levels of testosterone (T)[3-5], cortisol (C)[6,7] bioactive luteinizing hormone (bLH)[8] and prolactin (PRL)[8]. It is not known whether the photoperiodicity of environmental light plays a role in determining these daily rhythms. Such information would be of pertinent relevance to the better evaluation of any experimental procedure aimed at altering the endocrine function of rhesus monkeys. The need for such information becomes obvious in view of monkeys constituting an important experimental animal model in the preclinical evaluation of drugs and contraceptives.

Materials and methods. The present studies were carried out in 6 adult male rhesus monkeys (9-14 kg, b.wt) maintained

Table 1. Characteristics of RIA of T, C, PRL and bioassay of LH

Hormone	No. of assays	% coefficient of variation Intrassay	Interassay	Sensitivity
T	22	3.62	6.42	10pg
C	18	6.50	11.38	50pg
bLH	19	8.30	13.52	2ng
PRL	8	4.62	6.89	10μIU

Table 2. Mean levels (± SEM) of hormones in blood samples taken during day and night from adult male rhesus monkeys kept under either 12 h light: 12 h dark schedule or constant light

Hormones	12 h light: 12 h dark Day	Night	Constant light Day	Night
T (nmole/l)	28.11± 4.23	72.82± 4.84***	28.96± 2.08	65.14± 3.30***
C (nmole/l)	817.40±24.20	366.80±21.10***	779.10±34.11	563.12±28.87***
bLH (μg/ml)	1.46± 0.09	2.79± 0.07***	1.58± 0.05	2.46± 0.28**
PRL (mIU/l)	326.51±23.39	522.11±32.12**	362.50±37.71	478.60±44.00*

*$p<0.05$; **$p<0.005$; ***$p<0.001$. Comparisons between day and night levels done by Student's t-test[13].

in the Primate Research Facility of this Institute[9] to determine if the marked differences between the day and night levels of serum T, C, bLH and PRL observed in monkeys kept under controlled light:dark schedule are abolished by exposing the same animals to constant illumination. The monkeys were individually caged and housed in a room measuring 450 × 540 cm. Environmental light was provided by 6 fluorescent tube-lights of 40 W each located at a height of 180 cm from the cage. A 12 h light:12 h dark schedule was maintained by switching on the lights at 06.00 h and switching off at 18.00 h. After taking venous blood samples (5 ml each) from each animal at 12-h intervals (at 09.00 h and 21.00 h) for 5 days the animals were exposed to constant light for a period of 15 days. The blood sampling was repeated as described above for the next 5 days. These sampling times were chosen on the basis of our previous studies[10] which indicated that in most instances the marked differences between the day and night levels of these 4 hormones are observed in blood samples taken at these times of the day. The serum was stored at −20 °C until levels of T,C and PRL were estimated by specific radioimmunoassays (RIA) using reagents supplied by the World Health Organization under their Quality Control Programme[11,12]. Serum levels of bLH were estimated by using an in vitro bioassay system[8].

The RIA data was analyzed by logit-log transformation using Rodbards unweighted method on a Hewlett-Packard Programmable Calculator (Model No. 9831).

Statistical analysis between mean levels of hormones in day and night samples were determined by paired t-test[13]. The difference in hormonal levels between the day and night samples in monkeys exposed to the 2 different environmental lighting conditions as well as differences within a group during the 5 day of sampling as analyzed by 1-way analysis of variance[13].

Results and discussion. The results of the present study are summarized in the figure and table 2. The data clearly indicate that the pattern of day and night differences in the circulating levels of T,C,PRL,bLH observed in monkeys kept under 12 h light:12 h dark schedule is not markedly altered by exposing monkeys to constant illumination. A similar finding has recently been reported by other investigators[14] who found that exposure of rhesus monkeys to constant illumination does not alter the diurnal rhythms of C, while changes in the photoperiodicity of environmental light brings about immediate and marked changes in the circadian rhythms of circulating levels of melatonin. These findings suggest that the photoperiodicity of environmental light may not be the main determinant for the occurrence of daily diurnal rhythms observed in the circulating levels of T,C,PRL and bLH in rhesus monkeys. It is possible that the diurnal rhythms of the 4 hormones studied are either free-running and are regulated by an internal Zeitgeber or that these rhythms are cued-in by other extraneous factors which need to be identified. Further studies need to be carried out to elucidate the neuroendocrine mecanisms(s) and pathways regulating the diurnal rhythms in this primate species. However, it remains to be shown if chronic exposure of monkeys to constant illumination for periods in excess of 15 days will alter the diurnal rhythms.

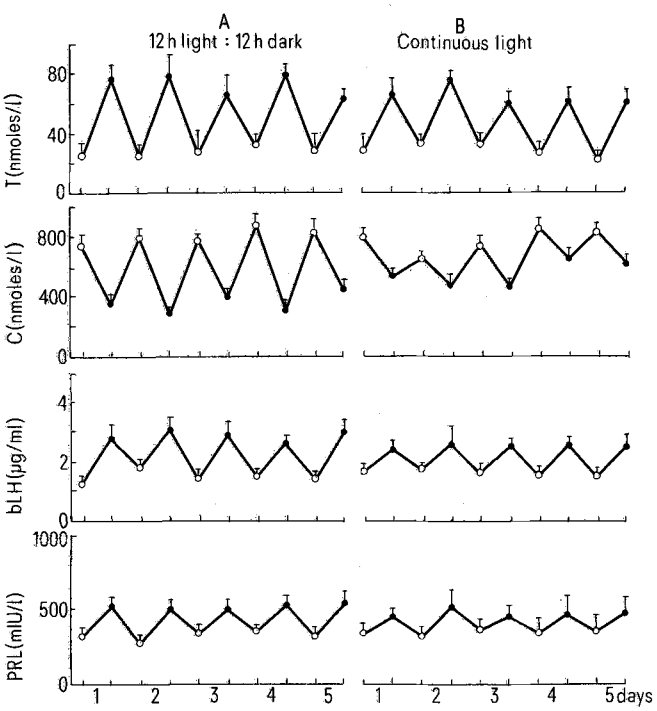
Table 3 shows the ratios of night vs day levels of the different hormones studied in monkeys kept under the 2 different lighting conditions. The marked differences in the ratios obtained for C,PRL and bLH between animals kept under the 2 lighting conditions suggest that exposure of animals to constant illumination may result in the alteration of the total amount of these hormones produced during a 24 h period but may not affect their diurnal rhythmicity. Comparison of hormonal levels in blood samples taken at more frequent intervals during a 24-h period between animals exposed to controlled light and constant illumination may resolve this issue and also indicate whether or not there is a temporal shift in the maxima but without a change in periodicity.

The results of the present studies, which clearly indicate the differences between day and night levels of T in serum persist in rhesus monkeys exposed to constant light, are different from the data obtained in bonnet monkeys which do not show the high nocturnal levels following their exposure to constant light for periods in excess of 72 h[15]. The reason for this marked species difference in the responses to environmental light needs to be further studied.

Table 3. Night vs day ratios between mean levels (±SEM) of different hormones in adult male rhesus monkeys kept under 12 h light. 12 h dark schedule or constant light

Hormone	12 h light: 12 h dark	Continuous light
T	2.60±0.17	2.27±0.15
C	0.45±0.04	0.72±0.41***
bLH	1.93±0.11	1.55±0.05*
PRL	1.68±0.03	1.33±0.08**

*p<0.025; **p<0.005; ***p<0.001. Comparisons between ratios done by student's t-test[13].



Mean levels (vertical bars: SEM) of T,C,PRL and bLH in the 12-h blood samples taken daily for 5 days during the day (open circles) and at night (closed circles) in the same group of 6 adult rhesus monkeys kept under 12 h light:12 h dark conditions or under constant illumination for 15 days. No marked differences could be observed between monkeys kept under the 2 different lighting conditions in the pattern of differences between day and night levels of the different hormones studied.

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Effect of oestradiol on RNA polymerase of foetal guinea-pig uterus

F. Laure and J.R. Pasqualini

C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26 boulevard Brune, F-75014 Paris (France), November 2, 1981

Summary. The administration of 10 µg oestradiol to the foetus of the guinea-pig (55-65 days of gestation) causes an increase in RNA polymerase I and II activities in the nuclei of the foetal uterus. RNA polymerase I activity increased by 4 times above the control values after 120 min ($p < 0.001$) whereas RNA polymerase II activity increased more rapidly, reaching 2.5 times the control levels at 30 min and 4 times by 120 min after treatment ($p < 0.01$).

In previous studies, it has been established that administration of oestradiol increases uterine wet weight[1] and provokes a stimulation of progesterone receptors[2] in the foetal uterus of the guinea-pig. Futhermore, these 2 parameters are correlated with the translocation and nuclear retention of oestradiol receptors[3]. It was also demonstrated that the foetal uterus responds to oestradiol by an intensification of nucleosomal histone acetylation[4] as well as by an increase in the incorporation of ^{3}H-leucine into acid-insoluble proteins[5]. Since several oestrogenic effects appear to be correlated with the increase in RNA polymerase activities during extrauterine life[6,7], it was interesting to investigate the effect of oestradiol on the RNA polymerase activities in the foetal uterus.

Hartley albino guinea-pigs of 55-65 days of gestation were purchased from a commercial breeder (Hiblot, Vigneux-sous-Montmedy, Meuse, France). Animals were anesthetized with 5% (w/v) pentobarbital - 0.006% (w/v) atropine and submitted to laparotomy. Each female foetus was injected s.c., in situ, with 10 µg of oestradiol in 10% ethanol-saline solution. The controls received vehicle alone. The foetuses were replaced in the abdomen of the mother and at different times (30, 60, 120, 240 min) removed and decapitated. The foetal uteri were then excised and stripped of fat. Tissues were homogenized in 3-4 ml of 0.25 M sucrose in TKM buffer (0.25 M sucrose - 0.05 M Tris-HCl - 0.025 M KCl - 0.005 M $MgCl_2$, pH 7.4) and centrifuged at 900×g for 10 min. The 900×g pellet was washed with 0.4 M sucrose in TKM buffer (1-2 ml) and centrifuged at 900×g. Finally, this pellet was homogenized in 2 M sucrose in TKM buffer (6 ml), layered on an equal volume of the same solution and centrifuged at 250,000×g for 60 min to obtain the purified nuclei.

The pellet containing the purified nuclei was resuspended in a small volume (1-2 ml) of TEDG buffer (0.05 M Tris-HCl - 0.0001 M EDTA - 0.0005 M dithiothreitol - 25% (v/v) glycerol, pH 7.9) for measurement of endogenous RNA polymerase activities. These activities were evaluated essentially by incubation at 37 °C for 30 min of an aliquot (0.05 ml) of the nuclear suspension with a same volume of reaction mixture containing 0.075 µmoles of ATP, CTP, GTP and 1 µCi (0.0005 µmoles) of ^{3}H-UTP (sp.act. 2 Ci/mmole, Amersham, Versailles, France) in either low ionic strength medium in the presence of Mg^{2+} (0.2 µmoles of $MgCl_2$) plus α-amanitin (1µg) for the evaluation of the RNA polymerase I activity, or high ionic strength medium in the presence of Mn^{2+} (0.2 µmoles of $MnCl_2$) for the measurement of RNA polymerase II activity, according to the method described by Borthwick and Smellie[8]. The enzymatic reaction was stopped by immersing the samples in crushed ice and an aliquot of each incubation was spotted on filter paper discs (Whatman GF/A 2.5 cm) and washed as described[8] with different solvents (10 and 5% (w/v) trichloracetic acid containing 1% (w/v) tetrasodium pyrophosphate, alcohol, alcohol-ether, ether). The discs were placed directly in a counting vial and, after drying, were treated for several hours with 0.5 ml of Soluene 350 (Packard Instruments S.A., Rungis, France). The incorporated radioactivity was measured in 3 ml scintillation fluid (PPO 0.5%-POPOP 0.01% (w/v) in toluene). Protein was measured according to the method of Lowry et al.[9] and the DNA determinations were carried out by the method of Burton[10].

Figure a shows that within the 1st hour after administration of 10 µg oestradiol to the foetus, the RNA polymerase I activity was not significantly different from control values, but by 120 min it was 4 times higher. Thereafter, the activity remained constant between 120 and 240 min. Figure b shows that the RNA polymerase II activity increased rapidly to reach 2.5 times the control values after