# INTRACELLULAR DISTRIBUTION OF ESTRADIOL AND ESTROGEN BINDING SITES IN THE UTERUS AND OVIDUCTS OF THE GREEN MONKEY *(Cercopithecus griseus)*

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UDC 612.662-08:[612.627:612.621.31

KEY WORDS: estrogen reception; green monkey; menstrual cycle; uterus; oviducts.

Research into the reproductive system of primates has intensified steadily in recent years because these animals are the most adequate model with which to study the corresponding system in man. The reproductive system of several species of macaques *(Macaca)* [9] and baboons *(Papio)* [8, 11] has now been studied in detail. Data on the state of the reproductive system of monkeys (guenons) of the *Cercopithecus* genus, which like *Macaca* and *Papio*, belong to the superfamily (Cercopithecoidea) of the Catarrhini suborder, are still scanty [2, 15]. The green monkey *(Cercopithecus griseus)* is a species that reproduces successfully under standard animal house conditions in the temperate geographic zone. The results of preliminary investigations on this species have shown that with respect to the duration of its menstrual cycle [3], the character of excretion of sex steroids [8], physicochemical parameters of interaction of estrogens with estrogen binding sites (EBS) in the cytosol of the uterus and oviducts [1], the reproductive system of monkeys of this species closely resemble that in man. No information on the concentrations of sex steroids and EBS in the uterus and oviducts of *C. griseus* could be found.

This paper describes a comparative investigation of changes in estradiol ($E_2$) and progesterone (P) concentrations in the blood plasma and concentrations of $E_2$ and EBS in the uterus and oviducts of *C. griseus* in the course of the menstrual cycle.

## EXPERIMENTAL METHOD

A 0.25 M aqueous solution of sucrose, 10 mM Tris-HCl buffer containing 1.5 mM EDTA (pH 7.4), a 0.1M phosphate buffer, containing 0.01% and 0.1% gelatin (pH 7.2), antisera against $E_2$-17β and P (Steranti), labeled $^3$H-$E_2$-17β and $^3$H-P (specific radioactivty 104 and 96 Ci/mmole respectively, from Amersham Corporation, England), and their unlabeled analogs (from Calbiochem, USA), 0.5, 1, and 5% suspensions of activated charcoal (Norit A) in buffer with the addition of 0.1% gelatin, and scintillation fluid consisting of 0.5% PPO and 0.05% POPOP in toluene, were used. Mature female green monkeys in certain phases of the menstrual cycle were killed by total exsanguination under pentobarbital anesthesia. The phase of the cycle was then confirmed by morphological investigations of the ovaries [3]. Blood was taken from the jugular vein and the plasma kept at −20°C. The uterus and oviducts were removed and transferred into sucrose solutions at 0°C. Cytosol and nuclear fractions were obtained as described previously [5]. The DNA concentration in the nuclear fractions was determined by the method in [7]. Concentrations of $E_2$ and P in the blood plasma and $E_2$ in the cytosol and nuclear fraction of the uterus and oviducts were measured by radioimmunoassay [4]. The concentration of EBS in the cytosol and nuclear fraction was determined by ligand exchange methods [6, 10]. The dissociation equilibrium constant ($K_d$) of interaction between $^3$H-$E_2$-17β and cytoplasmic EBS was determined by Scatchard's method [14]. The standard error of the means of the parameters listed above did not exceed 15%.

## EXPERIMENTAL RESULTS

The lowest values of plasma $E_2$ concentrations in female green monkeys during the menstrual cycle were found in the early follicular and late lutein phases (Fig. 1A). The highest

Laboratory of Endocrinology, All-Union Research Center for Maternal and Child Care, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 424-427, October, 1985. Original article submitted December 21, 1984.

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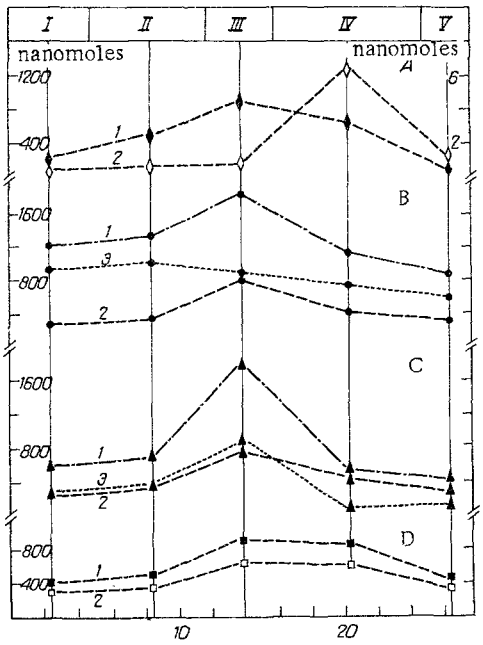


Fig. 1. Diagrams of relations between concentrations of $E_2$ and P in the blood plasma and $E_2$ and EBS in the cytosol and nuclear fraction of the green monkey uterus during the menstrual cycle. Here and in Fig. 2 — abscissa: top — phases, bottom — days of cycle. A) In blood plasma: 1) $E_2$ (in pM), 2) P (in nM); B) in cytosol (in fmoles/mg DNA): 1) total $E_2$, 2) bound $E_2$, 3) free $E_2$; C) in cytosol (in fmoles/mg DNA): 1) total EBS, 2) bound EBS, 3) free EBS; D) early follicular phase (n = 4), II) middle follicular phase (n = 5), III) periovulatory period (n = 10), IV) middle lutein phase (n = 6), V) late lutein phase (n = 5).

$E_2$ concentration, about 5 times higher than the basal level, was found in the periovulatory period (12th-14th day of the menstrual cycle). The $E_2$ concentration in the middle of the lutein phase was indistinguisable from this value. The maximal blood P concentration was observed in the middle of the lutein phase. It can be concluded after summarizing these results and the morphological data [10] that the trend of changes in the plasma $E_2$ and P concentrations of green monkeys during the menstrual cycle does not differ in principle from that of Afro-Asian monkeys and man [8, 9, 13].

Analysis of the data on the $E_2$ and EBS concentrations in the various subcellular fractions of the uterus (Fig. 1, B-D) revealed the following general principles. The concentration of free $E_2$ in the cytosol remained approximately constant during the menstrual cycle. For all other parameters, their values in the periovulatory period were characteristically higher than that in the early follicular and late lutein phase. Incidentally, the periovulatory "rise" of the values of these parameters was less marked than corresponding increase in the plasma $E_2$ concentration. The concentration of total (free + bound with EBS) $E_2$ in the cytosol and of $E_2$ and EBS in the nuclear fraction in the periovulatory period was the same as in the middle of the lutein phase. Compared with the periovulatory period, a significant decrease was observed in the levels of bound $E_2$, and of total (free + occupied, i.e., bound with endogenous estrogens), free, and occupied EBS in the middle of the lutein phase. Changes of a similar character in the concentrations of cytoplasmic and nuclear EBS in different phases of the menstrual cycle also have been found in the uterus of *Macaca rhesus* [12].

Unlike the uterus, in the cytosol of the oviducts (Fig. 2A-C) the concentrations of total and bound $E_2$ and also of occupied EBS did not change during the menstrual cycle. In the case of $E_2$ and EBS in the nuclear fraction and total EBS in the cytosol, a small but significant ($P < 0.05$) increase in their concentrations was found in the periovulatory period compared with these same parameters in the follicular and lutein phases.

Definite information on regulation of the molecular mechanisms of action of estrogen can be obtained by analysis of relations between the $E_2$ and EBS concentrations in the various subcellular fractions of the green monkey uterus during the menstrual cycle. The concentration of total $E_2$ in the cytoplasm of the uterus in the follicular phase was twice as high as

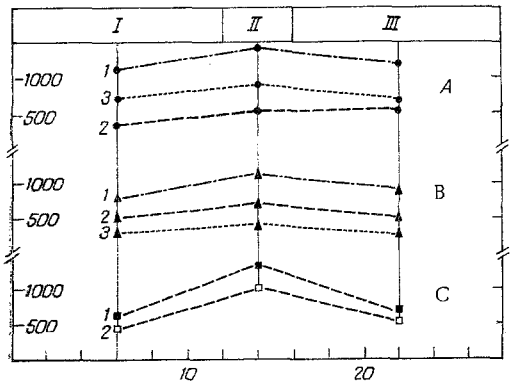


Fig. 2. Diagrams of relations between $E_2$ and EBS concentrations in cytosol and nuclear fraction of oviducts of green monkey during the menstrual cycle. A) $E_2$ in cytosol (in fmoles/mg DNA): 1) total, 2) bound, 3) free; B) EBS in cytosol (in fmoles/mg DNA): 1) total, 2) bound, 3) free; C) in nuclear fraction: 1) $E_2$ (in fmoles/mg DNA), 2) EBS (in fmoles/mg DNA). I) follicular phase (n = 9), II) periovulatory period (n = 10), III) lutein phase (n = 11).

the total EBS concentration, but in the periovulatory period, the values of these parameters did not differ significantly. The fraction of occupied EBS during these phases, moreover, was about 50% of the total, and the concentration of free $E_2$ and, consequently, the value of $K_d$ were constant during the first half of the cycle (Fig. 1B, C). These relationships are in strict accordance with the law of mass action for bimolecular interaction in a state of equilibrium.

In the middle and late lutein phase, despite no change in the ratio of total $E_2$ and EBS in the cytosol of the uterus, the number of occupied EBS was 82% and 74% of the total respectively. It can be tentatively suggested that the increase in the proportion of occupied EBS was due to an increase in activity of estrogen–receptor interaction in this period of the menstrual cycle. This hypothesis is confirmed by the significant ($P < 0.01$) reduction by half in the mean value of $K_d$ in the lutein phase ($0.36 \pm 0.03$ nM, n = 11) compared with that in the follicular phase and the periovulatory period ($0.76 \pm 0.08$ nM, n = 19).

The absence of differences between concentrations of bound $E_2$ and occupied EBS in the cytosol of the uterus and oviducts is evidence that endogenous $E_2$ in the target cell is in fact associated with EBS. In the uterus and oviducts the mean value of relations between concentrations of occupied cytoplasmic EBS and concentrations of nuclear EBS, calculated for each tissue specimen in the uterus and oviducts, was close to 1 ($1.1 \pm 0.2$; n = 30). This is evidently indirect evidence of the absence of any additional intracellular mechanisms affecting translocation of activated cytoplasmic estrogen–receptor complexes into the nucleus.

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# PHYLOGENETIC AND ONTOGENETIC ASPECTS OF LIPID PEROXIDATION IN THE VERTEBRATE RETINA

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UDC 617.735-007.23-092-07:617.735-008.939.15-074

KEY WORDS: lipid peroxidation; phylogeny; ontogeny; malonic dialdehyde; glutathione peroxidase.

Investigations of free-radical oxidation of lipids in biological membranes have shown that lipid peroxidation (LPO) products participate in various physiological processes (pinocytosis, phagocytosis, incorporation of iodine into thyroxine [13-15], regulation of membrane permeability, oxidative phosphorylation, and so on [2, 12]). It has also been shown that uncompensated activation of LPO plays an important, and sometimes decisive, role in the pathogenesis of certain diseases [2].

Having regard to the urgency of this problem, the role of LPO in visual function has been the subject of intensive study in recent years. It has been shown, in particular, that the action of visible light increases the rate of LPO in the photoreceptor membranes of the frog and pollock [4, 6, 8]. However, there have been very few studies of phylogenetic and ontogenetic aspects of LPO in the retina.

In the investigation described below LPO activity was studied in the vertebrate retina during phylogeny and ontogeny.

## EXPERIMENTAL METHOD

Retinas were obtained from representatives of various classes of vertebrates: fishes (carp), amphibians (frog), reptiles (turtle), birds (pigeon), and mammals (guinea pig, rabbit). In experiments with dark-adapted animals (2 h) all operations were conducted in weak red light, and with light adapted animals, in daylight. The retina was illuminated by means of an incandescent lamp (1200 lx, 30 min).

The ontogenetic studies were conducted on retinas of chick embryos and newborn rabbits. The chick embryos were obtained from poultry factories in light- and heat-proof boxes. At the time of decapitation the embryos were alive.

The intensity of LPO was judged from changes in concentrations of hydroperoxides [3] and malonic dialdehyde (MDA) [2]. Glutathione peroxidase (GP) activity in retinal homogenates was determined by known methods [5, 11] and the protein concentration by the biuret reaction [1].

## EXPERIMENTAL RESULTS

Retinas of dark-adapted representatives of different classes of vertebrates differed in their MDA and hydroperoxide concentrations (Fig. 1). It will be clear from Fig. 1 that the intensity of LPO in the dark-adapted retina decreased appreciably in order from fish to mammals (the pigeons were an exception). For example, whereas the MDA concentration in the carp retina was 0.880 ± 0.085 nmole/mg protein, in the rabbit retina it was 0.450 ± 0.040 nmole/mg protein.

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