the cytosol fraction, the incubates were treated with Dextran-coated charcoal suspension according to Beato and Feigelson[12]. The mixture was centrifuged for 10 min at 3,000 rpm at 4 °C. To the supernatant obtained, the phase combining system (PCS) scintillator (Amersham) diluted with toluene (PCS: toluene, 2:1 v/v) was added. For the nuclear fraction, the incubate was centrifuged at 800 × g for 10 min. The pellet obtained was washed with 0.25 M sucrose buffer followed by centrifugation at 800 × g for 10 min. This procedure was repeated twice. The washed pellet was solubilized with 0.4 ml 5 M urea-2 M NaCl with vigorous vortexing, and then the mixture was kept overnight at 0-2 °C. To the extract was added PCS scintillator diluted with toluene. The radioactivity was analyzed as described[8].

Results and discussion. The testosterone treatment decreased the initial high level of cytosol [^{3}H] testosterone-binding sites (figure 1) and contrarily, it increased substantially the initial low level of nuclear [^{3}H] testosterone-binding sites (figure 2). As the binding sites of the component which appeared in the nuclei were occupied by the non-radioactive steroid, the exchange assay was employed for the measurement of occupied receptors. The exchange assay has been used for the measurement of several steroid hormone receptors and provides a method for the evaluation of cytosol and nuclear receptors[13].

The distribution of binding sites between the cytosol and the nucleus suggested that the binding sites which appeared in the nuclei following the injection of testosterone are related to the disappearance of binding sites from the cytosol. Such a pattern of distribution and the appearance of the binding sites occupied by injected testosterone are consistent with the possibility that the androgen-specific binding sites of rat liver are androgen receptors which can be translocated to the nucleus. The decrease in cytosol receptor is transient and the cytosol receptor replenishes within 1 h. This rapid recovery may partially be due to a process that involves receptor recycling from the nucleus. Although the fate of the receptor translocated into the nucleus is not well known, it may possibly be inactivated by nuclear protease[14] and it may re-enter the cytosol after it dissociates from testosterone. It was of interest to note that the finding obtained here is similar to the distribution of estrogen-binding sites in the cytosol and the nucleus of the liver 30 and 60 min after ethinyl estradiol treatment in female rats[15].

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Plasma corticosterone fluctuations during the oestrous cycle of the house mouse

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Summary. The circadian rhythm of corticosterone concentration in the mouse persists throughout the oestrous cycle, but concentrations are significantly elevated at proestrus and oestrus.

It has been shown that activity of the pituitary-adrenal axis varies with the oestrous cycle in the rat. Buckingham[1] and Raps[2] have shown that plasma corticosterone levels are higher both in the morning and evening of the day of pro-oestrus than at other times in the cycle, although there is no interruption in the normal circadian pattern of variation. However, Champlin[3] found no difference in plasma corticosterone at different stages of the cycle in mice. The purpose of this study was to re-examine the mouse for plasma corticosterone variations during the oestrous cycle.

Since Schwartz[4] has shown that 5-day cycling rats differ in their hormonal balance from 4-day cyclers only mice on 4-day cycles were used. These were induced by the presence of males, as 4-day cycles are otherwise uncommon in mice (Bingel and Schwartz[5]).

Materials and methods. 200 virgin TO mice (A. Tuck and Sons) were randomly assigned to 6 groups for blood sampling at 4-h intervals. They were housed individually, each with a male in an inner wire cage, given food and water ad libitum and maintained at 22 ± 1 °C on a 14:10 h light:dark cycle. The animals were left undisturbed for 2 weeks, then each was blood-sampled once, by retro-orbital puncture[6] after ether anaesthesia. Sampling, in a room adjacent to the animal room, was complete within 3 min of entry to the animal room. Only 3 entries per session were made; sessions were spread over 3 days. Heparinized blood was centrifuged within 45 min of collection, and the plasma frozen and stored at − 20 °C until assayed.

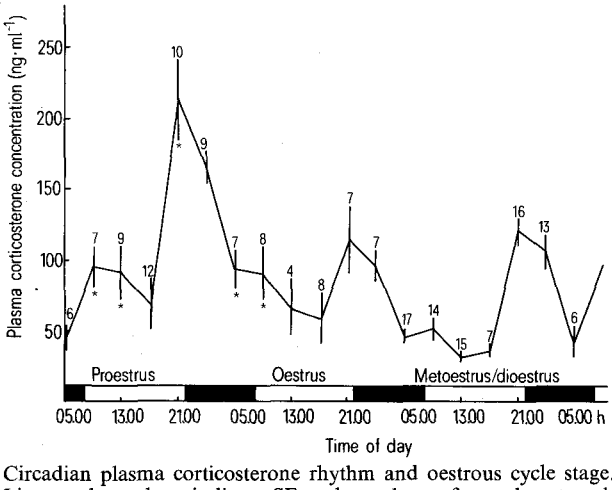
Corticosterone was determined by a radioimmunoassay similar to that used by Gross et al.[7] but using an initial

wash in 2,2,4-trimethyl pentane, extraction in ethyl acetate and rabbit anti-corticosterone-21-thyroglobulin serum supplied by Miles-Yeda. This anti-serum cross-reacts significantly (> 10%) only with progesterone, deoxycorticosterone (DOC) and 11-deoxycortisol (S), not with cortisol, testosterone, 17-hydroxyprogesterone, aldosterone, cortisone, oestrone, oestradiol 17-β, or oestriol. Since progesterone is removed by washing, and neither DOC nor S occur in significant quantities in mouse plasma[7], the assay has good specificity for corticosterone in this species. Recovery of corticosterone added to plasma averaged 94%. The relationship of the amount of corticosterone added to a plasma pool to amount estimated was linear over the range 10–400 ng/ml and the mean inter-assay variation was 9.1%. Sensitivity was 60 pg and the least detectable concentration of corticosterone was less than 5 ng/ml. One vaginal smear was taken 1–3 days before, and 5 consecutive daily smears immediately after blood sampling. Smears were dried, stained with Giemsa and staged according to Bingel and Schwarz[8]. Only 4-day cycling mice were included in the data. Values for metoestrus and dioestrus were combined and means compared by the Mann-Whitney U-test.

Results. The circadian rhythm of plasma corticosterone concentration was maintained through all stages of the oestrous cycle (see figure) with highest values always obtained at 21.00 h. Compared with values recorded at metoestrus and dioestrus, elevated corticosterone levels were recorded throughout the day and night of pro-estrus and only subsided late in the day of oestrus. Statistically significant differences are shown on the figure.

Discussion. Whilst most endocrine studies on the oestrous cycle have been carried out on rats, a few studies indicate that the mouse is similar[9,10]. The data presented here are in general agreement with those from the rat[1,2,11,12] showing corticosterone levels higher in the morning and evening of pro-oestrus than at other stages of the cycle, but our data show the elevation persisting well into the day of oestrus in mice.

The reason why some previous work with the mouse failed to show corticosterone elevation at pro-oestrus[3] may be the high variability of the oestrous cycle in mice[8]. This is particularly apparent in all female groups[13] with the absence of males and with daily vaginal smearing[5] where only a few mice show regular 4-day cycles.



Circadian plasma corticosterone rhythm and oestrous cycle stage. Lines and numbers indicate SE and numbers of samples at each point. Asterisks indicate statistically significant differences from the corresponding values for metoestrus and dioestrus (combined): p-values range from 0.025 to 0.001.

Oestrogen may be the key to the increase in corticosterone at pro-oestrus. ACTH[5] and corticosterone[14] are depressed by ovariectomy and restored by oestradiol-17-β in rats. Oestrogen induces increased TSH and thyroxine secretion, which in turn raise levels of corticosterone binding globulin (CBG)[15]. As the corticosterone-ACTH feedback operates only on unbound corticosterone[16] such a rise in CBG can lead to increased ACTH secretion, elevating plasma corticosterone. Oestradiol is high during the morning of pro-oestrus in the 4 day cycling rat[17] and TSH and thyroxine levels rise in the late morning and early afternoon[1,18]. It is not known whether there is a rise in CBG at pro-oestrus, but it has been shown that CBG levels can fluctuate daily in the male rat, and are higher in the female[19].

Phillips and Poolsanguan[11] have argued that corticosterone elevation at pro-oestrus in the rat is biphasic, the morning rise due to high oestrogen and low progesterone; the afternoon elevation due to LH[17] which has a stimulatory effect on the adrenal both in vitro and in vivo[20]. However, Buckingham et al.[1] have shown a peak of ACTH in the afternoon of pro-oestrus, higher than at other stages of the cycle, implying that the afternoon increase of corticosterone is mediated by ACTH. This extension of the pro-oestrus elevation of corticosterone reported here may reflect a more prolonged secretion of oestrogen which Bingel and Schwartz[8] have suggested occurs in the mouse.

The functional significance of increased corticosterone levels at pro-oestrus is as yet unclear. It is noted that the adrenal has been implicated in the timing of ovulation[21] and that an irregular corticosterone rhythm is correlated with irregular cycles in rats[22].

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