preliminary results show that, similarly to mouse lymphocytes, the majority of human T-cells are characterized by a round shape and a high nucleoplasmic ratio. Po-anti-HTLA conjugate was found to be irregularly distributed on the plasma membrane, in small spots, contrasting with the continuous distribution of Po-Fab anti-Ig stain. Partial redistribution of HTLA does not account for this difference since cells were pre-fixed before addition of Po-anti-HTLA Ig. Moreover, no spontaneous capping could be evidenced by anti-HTLA serum unless a second antibody layer was added[13]. Direct identification of T-cells with specific Po-anti-HTLA conjugate will allow an extensive study of the ultrastructure of T-lymphocytes at different stages of maturation in various lymphoid tissues.

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Plasma Free and Total Tryptophan During the Oestrus Cycle, in Ovariectomized and in Male Rats

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Summary. Plasma free tryptophan was higher during prooestrus and early oestrus than at metoestrus or in ovariectomized or male rats. In contrast, total tryptophan was higher in ovariectomized and male rats than at any time in cycling females.

The rate-limiting step in the synthesis of 5-hydroxytryptamine (5-HT) appears to be tryptophan hydroxylation[1], the concentration of tryptophan (T) in the brain being much lower than the Michaelis constant of the enzyme for its substrate[2]. The availability of T in brain depends primarily on the rate of transport into the brain[3], but also on the availability of T in plasma in the free form, as most T is bound to plasma albumin[4]. Although under many experimental conditions changes in the amount of free T may influence brain T and 5HT turnover[5,6], changes in 5HT metabolism do not always lead to easily interpretable changes in free T[7], as would be expected in a 'closed loop' feedback system. The easy pharmacological[8,9] and dietary[10] manipulation of free T indicates that a study of changes in peripheral and central T metabolism during a 'natural' rhythm, such as the diurnal[11] or the oestrus cycle, may provide more information as to the regulatory role of free T in cerebral serotoninergic metabolism.

The present study reports changes in plasma free and total T at different times during the oestrus cycle. Parallel analyses of regional brain T, 5HT, catecholamines and monoamineoxidase activity were also carried out[12]. As a comparison to cycling rats, plasma free and total T was measured in ovariectomized and in male rats.

Experimental. Adult female albino Wistar rats (210–240 g) were maintained for at least 3 weeks in an animal room controlled for light (from 05.00 h to 19.00 h) and temperature (24 ± 1.5 °C), fed ad libitum, and observed by means of daily vaginal smears at 08.00 h for the presence of 2 consecutive 4-day oestrus cycles. Male rats (210–240 g) were studied after a similar 3 week period in the same light regimen, ovariectomized rats (250–280 g) 6–7 weeks after operation. Groups of 5 rats in the identical phase of their oestrus cycle were decapitated, and trunk blood collected in 1/10 volume 5% EDTA. Plasma was immediately separated. Free T was obtained by ultrafiltration of 4 ml plasma (combined from 2–3 rats) as previously described for human plasma[13]; both free and total tryptophan concentrations were determined fluorometrically[14].

Results and discussion. Under controlled conditions of light, temperature, and feeding ad libitum, both the gender and the endocrinological state of an animal were found to be associated with changes in free and total plasma T (Table).

A possible diurnal rhythm must be considered before defining the hormone-related changes. A preliminary investigation in male rats had indicated that plasma free T did not vary greatly between 10.00 h and 18.00 h, but doubled at night (light phase: 1.54 ± 0.08 µg/ml (mean ± SEM), $n = 6$; dark phase: 3.53 ± 0.68 µg/ml, $n = 7$; $p < 0.02$). A detailed study of the diurnal rhythm[11] has shown that free and total T in rats have a minimum at midday and a maximum at midnight. We have found a similar diurnal rhythm in free T in man[13].

Plasma tryptophan in various endocrinological states[a]

Endocrinological state		Free tryptophan (µg/ml plasma)	Free:total tryptophan (%)	Total tryptophan (µg/ml plasma)
Prooestrus	10.00 h (12)	2.70±0.33[c,g]	24.6±2.4[d,e]	10.77±0.61[d,e,g]
Prooestrus	15.00 h (6)	2.87±0.48[c,g]	22.9±3.5[c,e]	12.51±0.47[b,h]
Oestrus	10.00 h (6)	2.24±0.21[c]	25.0±1.7[d,f]	8.92±0.46[d,f]
Oestrus	15.00 h (10)	1.96±0.22	18.1±1.1[d]	10.67±0.74[d,e,g]
Metoestrus	10.00 h (9)	1.63±0.21	19.5±2.3[c]	8.54±0.59[d,f]
Ovariectomized	17.00 h (6)	1.92±0.12	14.5±1.4[b]	13.57±0.85
Male	17.00 h (8)	1.46±0.16	9.6±1.5	17.53±1.55

[a] Mean ± SEM for the number of determinations given in brackets. Significance calculated with Student's t-test: [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$ compared with male rats. [e] $p < 0.05$; [f] $p < 0.001$ compared with ovariectomized rats. [g] $p < 0.05$; [h] $p < 0.001$ compared with metoestrus.

Since the diurnal variation of free and total T are minimal between 10.00 h and 17.00 h, the changes shown in the Table may be attributed to the differing endocrinological states. The low free T and high total T in male rats (and ovariectomized rats) can be clearly contrasted with the high free T and low total T in prooestrus of cycling rats.

The mechanisms underlying these sex differences are probably complex. The lower total T in females may be attributed to a higher liver tryptophan pyrrolase activity[15]. Variations in plasma albumin in different endocrinological states, or the modification of binding capacity by gonadal hormones, are possible factors influencing free T in cycling females and in males.

A comparison of the free T changes, observed in diurnal and oestrus rhythms with cerebral 5HT metabolism, indicate certain parallels. High free T in plasma is found during the dark phase, together with high brain T and high 5HT turnover[11]; in the dark phase 5HT synthesis is decreased whereas release of newly synthesized 5HT is increased[16]. Lowest 5HT concentrations are also found in the dark phase[16]. Lowered cortical 5HT concentrations in the dark have even been found in rats entrained to a 1 h light – 1 h dark cycle[17].

Analogously, the high plasma free T observed on the day of prooestrus may reflect central serotoninergic metabolism similar to that occuring mainly at night. This postulated high 5HT release during early prooestrus (which we have observed only indirectly as a marked decrease in endogenous 5HT concentrations from 10.00 h to 15.00 h[12]) may play an important role in the events leading to the LH surge on the afternoon of prooestrus[18].

High plasma free T in cycling rats as compared with low free T in male rats may similarly reflect observed differences of significantly higher 5HT turnover in females than in males[19,20]. Further support for a sex-specific difference in cerebral 5HT metabolism comes from the observation (unpublished) that in female rats killed between 11.00 h and 17.00 h (the time of presumed minimum brain T), brain T was markedly higher than in male rats killed at 04.00 h (the time of presumed maximum brain T). These differences were particularly large in regions connected with cyclic neuroendocrine function – the preoptic region, anterior and medioventral hypothalamus.

Brain T and 5HT in most of the 18 regions analyzed during the oestrus cycle[12] showed a parallel pattern of marked changes from 10.00 h to 15.00 h, without parallel changes in plasma free T. These results indicate that plasma free T is not the sole regulator of 5HT metabolism, but that the brain transport mechanism for T probably plays a more important role[3].

Although plasma free T does not appear to be synchronized with central 5HT metabolism during the oestrus cycle, it could be postulated that low free T mirrors a functional state of high 5HT synthesis and high free T a state of high 5HT release. If this hypothesis can be verified by further animal studies, plasma free T levels may possibly constitute a valuable index of cerebral serotonin metabolism even in man. We have found high plasma free T in the premenstrual phase of healthy women[13] and also in post-menopausal endogenous depressed women (in preparation). A reliable interpretation of these results can only be made with a better understanding of the complex inter-relationships between peripheral and central T metabolism.

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Progesterone Formation and Metabolism by Rabbit Placenta in vitro

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Summary. 4-^{14}C-Progesterone and 4-^{14}C-pregnenolone are metabolized in vitro by rabbit placenta, at day 15 and 28 of gestation, exclusively to compounds reduced in ring A (5β) and at carbon 3 and 20.

In previous work from this laboratory, it was demonstrated that mouse and rat placental quarters are capable of metabolizing radioactive pregnenolone and progesterone to several ring A reduced metabolites and C_{19} steroids[2,3]. Estrogen formation could not be detected[2–5]. It was of interest to find out whether steroidogenesis in placental tissue of rabbit follows a similar pattern.

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