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 Further support for a sex-specific difference in cerebral 5HT metabolism comes from the observation (unpublished) that in female rats killed between 11.00 h and 17.00 h (the time of presumed minimum brain T), brain T was markedly higher than in male rats killed at 04.00 h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.

[1] A. T. B. Moir and D. J. Eccleston, J. Neurochem. *15*, 1093 (1968).
[2] E. Jéquier, D. S. Robinson, W. Lovenberg and A. Sjoerdsma, Biochem. Pharmac. *18*, 1071 (1969).
[3] M. Hamon, S. Bourgoin, Y. Morot-Gaudry, F. Héry and J. Glowinski, Adv. biochem. Psychopharmac. *11*, 153 (1974).
[4] R. H. McMenamy and J. L. Oncley, J. biol. Chem. *233*, 1436 (1958).
[5] A. Tagliamonte, G. Biggio, L. Vargiu and G. L. Gessa, Life Sci. *12*, part II, 277 (1973).
[6] P. J. Knott and G. Curzon, Nature, Lond. *239*, 452 (1972).
[7] G. L. Gessa, G. Biggio, F. Fadda, G. U. Corsini and A. Tagliamonte, J. Neurochem. *22*, 869 (1974).
[8] H. G. Smith and C. Lakatos, J. Pharm. Pharmac. *23*, 180 (1971).
[9] C. R. Sirtori, V. M. Adreoli and P. F. Spano, Clin. Pharmac. Ther. *15*, 219 (1974).
[10] B. K. Madras, E. L. Cohen, J. D. Fernstrom, F. Larin, H. N. Munro and R. J. Wurtman, Nature, Lond. *244*, 34 (1973).
[11] W. W. Morgan, J. J. Saldana, C. A. Yndo and J. F. Morgan, Brain Res. *84*, 75 (1975).
[12] W. Küng, E. Chappuis-Arndt and A. Wirz-Justice, Experientia *31*, 722 (1975).
[13] A. Wirz-Justice, W. Pühringer, G. Hole and R. Menzi, Pharmakopsychiat. *8*, 310 (1975).
[14] W. D. Denckla and H. K. Dewey, J. Lab. clin. Med. *69*, 160 (1967).
[15] G. Curzon, Br. J. Psychiat. *115*, 1367 (1969).
[16] F. Héry, E. Rouer and J. Glowinski, Brain. Res. *43*, 445 (1972).
[17] G. Hanselmann and A. Borbély, J. Neurochem., *26*, 951 (1976).
[18] C. Kordon and J. Glowinski, Neuropharmacology *11*, 153 (1972).
[19] E. L. Bliss in *Serotonin and Behavior* (Eds. J. Barchas and E. Usdin; Academic Press New York and London) 1973, p. 321.
[20] H. Bürki, unpublished observations.

Progesterone Formation and Metabolism by Rabbit Placenta in vitro

M. Marchut[1]

Department of Endocrinology, Institute of Pharmacology, Polish Academy of Sciences, 52 Ojcowska str., P–31–344 Krakow (Poland), 29 March 1976.

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.

[1] The author is grateful to Dr. R. Rembiesa for reviewing the manuscript.

[2] R. Rembiesa, M. Marchut and A. Warchoł, J. Steroid Biochem. *2*, 111 (1970).
[3] R. Rembiesa, M. Marchut and A. Warchoł, Steroids *19*, 65 (1972).
[4] L. Townsend and K. J. Ryan, Endocrinology *87*, 151 (1970).
[5] S. W. C. Chan and J. H. Leathem, Endocrinology *96*, 298 (1975).

Table I. Recrystallization to constant specific activity of radiometabolites synthesized from 4-^{14}C-progesterone and 4-^{14}C-pregnenolone by rabbit placenta in vitro

Steroid isolated	Substrate used	Specific activity (dpm/mg)				
		Calculated	Number of crystallization			
			1	2	3	4
5β-pregnan-3,20-dione	Progesterone	3365	3218[a]	3148	3109	
			3748	3182	3105	
3β-hydroxy-5β-pregnan-20-one		3037	2765	2575	2506	2472
			2807	2607	2533	2475
3α-hydroxy-5β-pregnan-20-one		1388	1437	1353	1295	1240
			1542	1406	1255	1238
3β,20α-dihydroxy-5β-pregnane		2037	865	457	384	382
			4679	1471	914	390
3α,20α-dihydroxy-5β-pregnane		1019	474	451	440	439
			3739	715	483	443
5β-pregnan-3,20-dione	Pregnenolone	1342	1405	1300	1308	
			1580	1390	1320	
progesterone		670	620	605	592	
			713	661	608	
3β-hydroxy-5β-pregnan-20-one		1288	1056	973	957	
			1674	1022	951	
pregnenolone		1548	1484	1453	1455	
			1678	1504	1470	

[a] Upper values are crystals and lower the corresponding mother liquors. The solvent systems used for recrystallization were: acetone: *n*-hexane, methylene dichloride: isooctane, acetone: water, ethanol 70%, methanol 70%.

This paper describes the in vitro metabolism of 4-^{14}C-progesterone to 5β-reduced metabolites by rabbit placenta on day 15 and 28 of gestation. In addition 4-^{14}C-pregnenolone conversion to progesterone and 5β-pregnane derivatives was reported.

Material and methods. White pregnant rabbits of Dutch strain were killed on day 15 or 28 of gestation. The placentae were removed immediately and each one was divided into 8 parts. Samples of 400 mg of tissue were preincubated for 30 min, in 5 ml of Krebs-Ringer bicarbonate buffer at 37 °C under 95% O_2–5% CO_2 atmosphere in a shaking incubator. After that, the medium was discarded and replaced with fresh buffer containing 0.2 μCi of 4-^{14}C-pregnenolone (spec. activity 51 μCi/mmol), or 0.2 μCi of 4-^{14}C-progesterone (spec. activity 60 μCi/mmol) dissolved in a small volume of ethanol. After 2.5 h incubation, the tissue and medium were extracted 3 times with methylene dichloride. Following evaporation of the solvent, the residue was partitioned between toluene/*N* NaOH to yield a phenolic and neutral fraction. Purification and separation of the radioactive metabolites present in both fractions was accomplished by paper and thin layer chromatography. The radiometabolites were tentatively identified by identical mobilities with reference steroids and were further characterized by derivatives formation which included acetylation or oxidation with chromium trioxide[6]. Chemical identities were finally established by recrystallization to constant specific activities[7]. The radioactive metabolites were detected on chromatograms by means of automatic chromatogram scanner. Radioactivity was counted in a liquid scintillation spectrometer (Intertechnique, Paris, model SL 30).

Results. The average recovery of radioactivity from the incubations with 4-^{14}C-pregnenolone and 4-^{14}C-progesterone following methylene dichloride extraction was 72–78%. Partition of the methylene dichloride extracts be-

Table II. Percentage distribution of radioactivity in metabolites isolated following incubation of rabbit placenta with 4-^{14}C-progesterone and 4-^{14}C-pregnenolone at different stages of gestation

Metabolites isolated	Progesterone		Pregnenolone
	Day of gestation		
	15	28	15
Pregnenolone			68.60±1.72
Progesterone	7.32±0.66	0.87±0.05	2.43±0.81
Origin + unidentified polar material	3.95±0.53	2.41±0.09	13.95±1.03
3β,20α-dihydroxy-5β-pregnane + 3α,20α-dihydroxy-5β-pregnane	3.85±0.39	3.81±0.22	
3α-hydroxy-5β-pregnan-20-one	25.75±0.99	34.91±1.94	
3β-hydroxy-5β-pregnan-20-one	9.71±0.96	20.64±0.98	8.16±0.58
5β-pregnan-3,20-dione	48.66±1.45	36.68±0.92	6.87±0.53

The results are expressed as mean ± SEM, $n = 4$. The data represent % of total radioactivity recovered in each product and residual substrate after incubation and chromatographic separation.

tween toluene/N NaOH showed that over 99% of radioactivity was present in the neutral fractions and only 0.63–0.78% was associated with phenolic fractions. The presence of estrone and estradiol-17β was excluded in phenolic fractions from both precursors. Initial paper chromatography of the neutral metabolites from progesterone incubations (in cyclohexane: benzene (3:1)-propylene glycol system) resulted in the separation of the material into 4 zones of radioactivity. Zone 1 included: polar unidentified material; zone 2: 3α,20α-dihydroxy-5β-pregnane and 3β,20α-dihydroxy-5β-pregnane; zone 3: 3α-hydroxy-5β-pregnan-20-one and 3β-hydroxy-5β-pregnan-20-one; zone 4: unconverted progesterone and 5β-pregnan-3,20-dione. The crystallization data for the metabolites identified are presented in Table I. The formation of testosterone, 4-androsten-3,17-dione and 5β-androstane derivatives was excluded by crystallization study. As can be seen from Table II, progesterone was extensively reduced to 5β-pregnane derivatives by the placentae from both 15 and 28 day pregnant rabbits. It is also evident that the yield of pregnenolone conversion was much lower than that of progesterone. In the neutral fraction from pregnenolone incubations, progesterone was isolated. Besides, 3β-hydroxy-5β-pregnan-20-one and 5β-pregnan-3,20-dione were also identified (Table I).

Discussion. The present results show that reduction to 5β-pregnane derivatives is the only metabolic pathway of progesterone and pregnenolone in the placenta of mid-gestation and near term rabbits. Enzyme activities demonstrated by the isolation and identification of the pregnenolone and progesterone metabolites are: Δ^5-3β-hydroxysteroid dehydrogenase, Δ^5,Δ^4-isomerase, 5β-reductase, 3α-, 3β-, and 20α-hydroxysteroid dehydrogenases. Pregnenolone conversion to progesterone, shown in the present experiment, confirms the previous report of MATSUMOTO et al.[8]. The lack of estrogen synthesis in rabbit placenta has already been reported[9]. Stereospecific 5β-reduction in rabbit placenta is unique among short gestation species. Present results are in contrast to those reported in similar studies with mouse and rat placental quarters and homogenates, in which the the principal metabolites were shown to be saturated 5α-pregnane derivatives[2–4]. The most pronounced difference in the distribution of enzymatic activities in the placentae of various species appears to lie in the absence or presence of 17-hydroxylase and 17–20 lyase activity. The capacity for C_{19} steroid synthesis from C_{21} precursors could not be found in rabbit placenta, as was shown earlier in mouse and rat[2,3,5]. These observations emphasize once again the important differences which exist in the nature of the metabolites formed from the same precursors by the same organ from different mammalian species.

6 I. E. BUSH, *The Chromatography of Steroids* (Pergamon Press, Oxford 1961), p. 358.

7 L. R. AXELROD, C. MATTHIJSSEN, J. W. GOLDZIEHER and J. E. PULLIAM, Acta endocr., Copenh. (1965), Suppl. 99.

8 K. MATSUMOTO, G. YAMANE, H. ENDO, K. KOTOH and K. OKANO, Acta endocr., Copenh. *61*, 577 (1969).

9 L. AINSWORTH and K. RYAN, Endocrinology *79*, 875 (1966).

Pre- and Post-Partum Plasma Amine Oxidase Differences in the Rhesus Monkey (*Macaca mulatta*)[1]

D. E. REDMOND, JR.[2], J. BAULU and D. L. MURPHY

Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven (Connecticut 06510, USA) and the Laboratory of Clinical Science, N.I.M.H., and the Caribbean Primate Research Center at La Parguera (Puerto Rico), 16 March 1976.

Summary. Plasma amine oxidase activity increased from 23.4 nmol/ml/h during pregnancy to 49.5 nmol/ml/h during an extended post partum period in 10 rhesus monkeys. Comparison with non-pregnant control monkeys sampled at similar times indicated that the significant differences were in the extended post partum period.

Monoamine oxidase, a deaminating enzyme (MAO, monoamine: O_2 oxidoreductase deaminating EC.1.4.3.4) was first discovered by HARE[3] in 1928. Its major roles in deactivating and degrading monoamine neurotransmitters and amine metabolites, and in regulating amine synthesis via a feedback mechanism, have been extensively reviewed[4–7]. Various isoenzymes are found associated with mitochondria in body tissues, platelets, and nerve-ending preparations from brain[8–10]. A soluble enzyme found in the plasma has different co-factor requirements and substrate-inhibitor responses from the mitochondrial MAO's found in all other tissues. The plasma enzyme has the highest affinity for benzylamine[11,12], although tyramine has been the most frequently studied substrate. Previous investigators have observed lower plasma MAO activities in women taking synthetic progestin-containing pharmacologic agents than in age-matched controls[13]. One study found decreases in plasma MAO from an

1 We thank Dr. C. H. CONAWAY and Dr. J. D. LOY of the Caribbean Primate Research Center and J. G. VANDENBERGH and W. POST of the North Carolina Department of Mental Health for the use of their facilities and animals. We also acknowledge the technical assistance of C. H. DONNELLY and Ms. SUZANNE BAULU. This work was supported by a grant from Dr. H. C. ROBINSON, JR., through Yale University Medical School and by NIH Contract No. 71–2003.

2 Address reprint requests to: D. E. REDMOND, JR., Department of Psychiatry, 34 Park Street, New Haven, Connecticut 06510, USA.

3 M. L. C. HARE, Biochem. J. *22*, 986 (1928).

4 I. J. KOPIN, Pharmac. Rev. *16*, 179 (1964).

5 F. COSTA and P. GREENGARD, *Advances in Biochemical Psychopharmacology* (Raven Press, New York 1972), vol. 5.

6 N. WEINER and R. BJUR, in *Advances in Biochemical Psychopharmacology*, (Eds. F. COSTA and P. GREENGARD; Raven Press, New York 1972), vol. 5, p. 409.

7 D. L. MURPHY, C. H. DONNELLY, and M. BUCHSBAUM, in preparation (1976).

8 J. P. JOHNSTON, Biochem. Pharmac. *17*, 1285 (1968).

9 N. H. NEFF and C. GORIDIS, in *Advances in Biochemical Psychopharmacology* (Eds. F. COSTA and P. GREENGARD; Raven Press, New York 1972), vol. 5, p. 307.

10 C. H. DONNELLY and D. L. MURPHY, in preparation (1976).

11 C. M. MCEWEN, JR., J. biol. Chem. *240*, 2003 (1965).

12 C. M. MCEWEN, JR., in *Advances in Biochemical Psychopharmacology* (Eds. F. COSTA and P. GREENGARD; Raven Press, New York 1972), vol. 5, p. 151.

13 N. TRYDING, S. E. NILSSON, G. TIFVESSON, B. BERG, S. CARLSTROM, B. ELMFORS, and J. E. NILSSON, Scand. J. clin. Lab. Invest. *23*, 79 (1969).