

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-17 β 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: 3 α ,20 α -dihydroxy-5 β -pregnane and 3 β ,20 α -dihydroxy-5 β -pregnane; zone 3: 3 α -hydroxy-5 β -pregnan-20-one and 3 β -hydroxy-5 β -pregnan-20-one; zone 4: unconverted progesterone and 5 β -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 5 β -androstane derivatives was excluded by crystallization study. As can be seen from Table II, progesterone was extensively reduced to 5 β -pregnane derivatives by the placenta 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, 3 β -hydroxy-5 β -pregnan-20-one and 5 β -pregnan-3,20-dione were also identified (Table I).

Discussion. The present results show that reduction to 5 β -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 -3 β -hydroxysteroid dehydrogenase, Δ^5 , Δ^4 -isomerase, 5 β -reductase, 3 α -, 3 β -, and 20 α -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 reported⁹. Stereospecific 5 β -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 principal metabolites were shown to be saturated 5 α -pregnane derivatives²⁻⁴. The most pronounced difference in the distribution of enzymatic activities in the placenta of various species appears to lie in the absence or presence of 17-hydroxylase and 17–20 lyase activity. The capacity for C₁₉ steroid synthesis from C₂₁ 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.

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

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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: O₂ oxidoreductase deaminating EC.1.4.3.4) was first discovered by HARE³ 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 reviewed⁴⁻⁷. Various isoenzymes are found associated with mitochondria in body tissues, platelets, and nerve-ending preparations from brain⁸⁻¹⁰. 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 controls¹³. One study found decreases in plasma MAO from an

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average of 6 h before delivery to an average of 78 h after¹⁴. However, there was no difference between plasma MAO activity in 3rd trimester pregnant human females and controls in another study¹⁵. Recent studies have suggested the possibility that the tyramine-deaminating enzyme activities measured in the latter study after centrifugation at 1000–2000 *g* reflected residual particulate MAO activity from platelets^{16,17}. Consistent with this interpretation, the plasma MAO changes previously reported in association with the menstrual cycle in women^{15,18} were not found by BELMAKER et al.¹⁹ in plasma enzyme that had been centrifuged at 80,000 *g* to remove residual particulate enzyme activity. Changes were noted instead in the platelet MAO. We have replicated these findings in the female rhesus monkey during the menstrual cycle²⁰. The present study was undertaken to investigate the possible effects of pregnancy on plasma MAO activity in the rhesus monkey, using high speed centrifugation and specific inhibitors to remove possible contaminating mitochondrial enzyme.

Materials and methods. 10 healthy pregnant adult female rhesus monkeys (*Macaca mulatta*) were studied from the Caribbean Primate Research Center at La Parguera. Their mean census-determined age was 9.1 years (± 1.8 SEM). An equal number of non-pregnant females were studied as controls, aged 8.7 years (± 1.6 SEM). 16 of these animals were enclosed in $\frac{1}{2}$ or $\frac{1}{4}$ acre corrals where they were fed Purina monkey chow and fresh water at a single feeder ad libitum. 4 control monkeys were individually caged. Monkeys were caught in nets and hand restrained without anesthetics while 10 cm³ blood was drawn via a saphenous venipuncture into sodium heparinized syringes. The specimens were drawn on each animal during the latter part of pregnancy, after delivery, or at corresponding times of the year for controls, an average of approximately 4 months apart. Specimens were placed in acid citrate dextrose (ACD) preservative and cooled in an ice bath prior to preparation. The specimens were centrifuged at 2000 *g* for 30 + min at 4°C and plasma was removed and frozen on dry ice within 3 h of drawing. Plasma was stored at –20°C until analysis. Plasma MAO was determined using a modification of the method described by ROBINSON et al.²¹ using ¹⁴C-benzylamine (10^{–4} *M*) as substrate. Values are reported as nanomoles of deaminated product/ml plasma/h. A two-factor analysis of variance with repeated measures on one factor was used to analyze the data, after tests of the homogeneity of variances in the partitioned error terms. Tests of simple main effects completed the analysis after a significant AB interaction was found²².

Results. Plasma amine oxidase activity increased in each of the pregnant animals from the specimen during the pregnancy to the postdelivery specimen ($F = 19.1$, $p < 0.001$). The repeated measure was not significantly different in the controls ($F = 0.2$, N.S.). The prepartum mean was not significantly different from the corresponding time specimen for the controls ($F = 2.3$, N.S.), while the post-partum mean was significantly different ($F = 130$, $p < 0.001$) (Table).

Discussion. The differences in this study are between post partum MAO activities and corresponding activities from normal non-pregnant females without infants in that birth season, and between post partum values and the values from previous specimens during pregnancy in the same animals. Hormonal changes in the post-partum period or associated with nursing infants may be involved in these differences. Some socially-induced stress related to being the mother of a young infant in a primate social group might also be implicated, although we know of no behavioral data to support this possibility.

There is evidence from other studies that mitochondrial MAO activity in a variety of tissues is affected by the estrus cycle²³ or by pre-treatment with estradiol or progesterone²⁴. We have noted effects of the menstrual cycle and of ovariectomy on platelet MAO in rhesus monkeys, using tyramine as substrate, but found no changes in plasma amine oxidase, using benzylamine²⁰. The results reported here appear to support the finding of no difference in plasma MAO activity, measured with tyramine as substrate, in pregnant human females and controls by BRIGGS and BRIGGS¹⁵. Our results are not consistent with the findings of TRYDING et al.¹³ who found reduced plasma MAO activity in human females taking synthetic progestin-containing pharmacologic agents, whether amine oxidase activity was measured with benzylamine or tyramine as substrate. The small differences in the op-

Plasma MAO activity (nmoles of benzylamine oxidized/ml plasma/h) in paired specimens from 10 female rhesus monkeys and corresponding specimens from non-pregnant controls

Monkey No.	Mothers		Monkey No.	Controls	
	Pre-	Post-partum		Spec. 1	Spec. 2
095	7.1	16.7	303	25.5	23.3
231	10.4	17.3	075	13.0	21.5
R65	23.1	31.4	288	5.8	13.9
060	39.5	121.3	08D	21.4	31.9
0Y6	17.8	50.8	09G	48.2	29.4
0Z1	13.0	19.3	09C	24.7	24.3
143	67.2	81.5	0L9	22.9	15.0
685	34.3	85.5	283	36.0	30.8
0X3	11.7	25.3	309	43.1	28.8
834	9.8	45.7	241	27.7	18.7
Mean	23.4	49.5		26.8	23.8
S.D.	18.8	35.6		12.9	6.5
S.E.M.	6.0	11.3		4.1	2.0
Mean No. days from Delivery					
	–82.3	+30.7			
S.E.M.	8.4	7.9			

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posite direction found in the study by GILMORE et al.¹⁴ were from samples drawn from 6 h before to 134 h after delivery, a short time period for which we have no comparable data.

The significance of plasma amine oxidase activity and its relationships to brain or sympathetic monoamine function is not known. Differences in enzymes related to the monoamine systems are of interest because of possible relevance to the behavioral changes associated with the post partum period in humans such as the puerperal psychoses which have been reported since Hippocrates²⁵. There is a disagreement as to whether these psychoses represent distinct nosological entities or whether factors associated with the puerperal period precipitate the expression of episodes of schizophrenic or manic-depressive psychosis²⁶⁻³⁰, both of which have been associated with changes in other monoamine enzyme activities³¹⁻³⁷.

Thyroid Hormone in Serum of Fetal Calf and Pregnant Cow During the Last Trimester of Pregnancy

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Summary. Considerably higher thyroxine and triiodothyronine concentrations in sera of bovine fetuses than in maternal samples were found during the last trimester of pregnancy.

It was shown that mammalian fetal thyroid is active in utero and that negative feedback control appears to be functional before parturition^{4,5}. To know more about mother-fetus interrelation, we compared thyroid hormone content in sera of bovine fetuses and pregnant cows during the last third of pregnancy.

Material and methods. 23 pregnant Czech red-white cows in the 241-279th day of gestation were clinically and hematologically examined before experiment. They were submitted to high epidural anaesthesia with procain, the head of fetus was exteriorized, arteria carotis was cannulated and the fetus was exanguinated⁶. Samples of maternal blood were withdrawn during surgical procedure.

In maternal and fetal sera, thyroxine was determined by radioimmunological method⁷ with some modifications of FÖLDES⁸ and triiodothyronine was determined accord-

ing to NAUMAN and NAUMAN⁹. Thyroxine antibody was produced and kindly supplied by Dr. FÖLDES⁸, triiodothyronine antibody was the kind gift from Dr. NAUMAN⁹.

Results and discussion. The results are summarized in the Table. Both thyroid hormones, thyroxine and triiodothyronine are in at least twice as high in concentration in fetal sera as in maternal ones. Student's *t* and paired tests are highly significant. We did not find any correlation between thyroid hormone values in fetuses and mothers, between thyroid hormone content in sera and body weight of fetuses. We also did not find any tendency of thyroid hormone concentrations to change according to age of fetuses. In fetuses, unlike mothers, positive correlation between thyroxine and triiodothyronine was noted.

In the sheep fetus, high thyroxine secretion rate in comparison to maternal one was described^{10,11}. NA-

Thyroxine (T₄) and triiodothyronine (T₃) concentrations in maternal and fetal sera. BW, body weight; n.s., non-significant

	Mothers		Fetuses		P
	n	Means ± SE	n	Means ± SE	
T ₄ (nmol/l)	23	42.9 ± 3.3	23	109.4 ± 5.1	< 0.001
T ₃ (nmol/l)	19	0.50 ± 0.05	19	1.22 ± 0.15	< 0.01
Correlation coefficients					
T ₄ (mother:fetus)		+ 0.07767			n.s.
T ₃ (mother:fetus)		+ 0.21016			n.s.
Fetal					
T ₄ :BW			23	+ 0.20033	n.s.
T ₃ :BW			19	+ 0.00243	n.s.
T ₄ :T ₃			19	+ 0.45442	< 0.05
Maternal					
T ₄ :T ₃	19	- 0.42385			n.s.

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