Concentration of DHEA, c-AMP, and free estriol in maternal and cord plasma

| Parameter | Initial labour | During expulsion | 2 h after delivery | 48 h after delivery | Cord artery | Cord vein |
|---|---|---|---|---|---|---|
| DHEA (µg/100 ml) | | | | | | |
| Free | 2.7± 1.1 | 3.5± 0.9 | 3.3± 0.7 | 2.6± 1.5 | 8.7± 4.4 | 5.7± 2.2 |
| Sulfatide | 48.3± 20.4 | 51.3± 19.7 | 38.2± 14.5 | 40.8± 18.0 | 112.8± 47.5 | 73.4± 34.2 |
| Sulfate | 32.9± 8.5 | 42.4± 14.4 | 19.0± 7.2 | 26.4± 8.3 | 80.4± 46.2 | 45.2± 6.7 |
| c-AMP (pMol/ml) | 11.2± 2.8 | 21.0± 6.4 | 16.0± 4.1 | 14.1± 4.7 | 31.4± 11.5 | 30.3± 12.2 |
| Estriol (ng/ml) | 10.2± 4.3 | 4.0± 1.9 | 2.0± 1.1 | 0.2± 0.09 | 5.7± 2.7 | 12.1± 6.7 |

nant women near term, the above values indicate a distinct increase in adrenocortical activity. The enhanced biosynthesis of primarily sulfoconjugated DHEA subsided after delivery, the levels of total DHEA falling to 60.5 µg/100 ml and 69.8 µg/100 ml 2 and 48 h resp. later. That the fetal adrenal provides a substantial portion of DHEA for placental biosynthesis of estriol[3] may be gathered from comparatively high concentrations of total DHEA in umbilical cord arterial plasma (201.9 µg/100 ml), the levels agreeing with those reported by SIMMER et al.[3]. Concerning the distribution of DHEA upon the fractions of free and sulfoconjugated steroids, the fraction of steroid sulfatides contained between 52.7% and 63.1% of assayed DHEA, followed by 31.4–43.6% in the fraction of steroid sulfates, and 3.2–5.5% in the fraction of free steroids. Although the instability of steroid sulfatides[4,5] may have affected these figures – in previous experiments the fraction of steroid sulfatides comprised roughly 80% of total DHEA in peripheral plasma of normal subjects – it can be concluded that the fetal adrenal also secretes DHEA sulfatide.

With regard to the assumed interrelationship between DHEA and c-AMP[8], the present results reveal a substantial rise of plasma c-AMP during the expulsion period, followed by a rapid decrease after delivery. Concomitant with the high concentrations of DHEA in umbilical cord arterial (or venous) plasma also elevated levels of c-AMP were obtained, supporting the contention that DHEA – especially as the sulfatide – exerts a definite influence upon plasma c-AMP. Regarding the nucleotide levels reported here, it should be pointed out that heparinized plasma was used throughout as in preceding investigations[8]. Hence, these concentrations cannot be compared with those gained with EDTA-treated plasma, which yields higher levels of c-AMP due to immediate inhibition of phosphodiesterase, as will be seen in a forthcoming communication.

When free estriol was determined in the various samples, a decline was observed in the expulsion period, which may be attributed to a reduced biosynthesis of placental estrogens. Whether an insufficient supply of $C_{19}$-steroid precursors is responsible for this decrease, or an impaired metabolic activity of placental tissue, remains to be seen. Within 48 h the maternal plasma concentration of free estriol had returned to non-pregnancy levels. In contrast to SCHILD et al.[13], who could not verify significant differences in the estriol content of umbilical cord arterial and venous blood, the higher levels of free estriol in the venous blood, shown in the Table, might very well hint at its placental origin.

[9] G. W. OERTEL, P. MENZEL and D. WENZEL, J. Steroid Biochem. *1*, 17 (1969).
[10] L. PENZES and G. W. OERTEL, Acta endocr. copenh. Suppl. *158*, 94 (1972).
[11] B. L. BROWN, J. D. M. ALBANO, R. P. EKINS and A. M. SCHERZI, Biochem. J. *121*, 561 (1971).
[12] R. GOEBEL and E. KUSS, Geburtsh. Frauenheilk. *34*, 329 (1974).
[13] W. SCHILD, K. SCHÜRHOLZ and A. SEUKEN, Arch. Gynäk. *198*, 136 (1963).

## Sexual Steroids and Monoamine Metabolism During Gestation[1]

SIMONE PARVEZ, A. RAZA-BUKHARI and H. PARVEZ

*Université de Paris, Centre d'Orsay, Laboratoire d'Endocrinologie, Bâtiment 491, F-91405 Orsay (France), 4 August 1975.*

*Summary.* The experiments show influence of progesterone and estradiol on regulation of enzymes of monoamine metabolism, MAO and COMT during pregnancy. Both the hormones inhibit enzymes MAO and COMT in the adrenals when determined at 0 h parturition. Estradiol has stronger inhibitory effect than progesterone. The results provide evidence for important endocrine implication during pregnancy for processes of monoamine regulation.

The role of sexual steroids in regulation of monoamine metabolism has been a subject of great interest recently. Hormones can modify the physiological disposition of labelled amines in rat uterus[2,3]. Progesterone increases monoamine oxidase (MAO) in the uterus during estrus[4,5]. Cyclic variations in progesterone production by adrenals and ovaries directly affect monoamine metabolism[6,7]. In the rat, estradiol inhibits MAO and progesterone seems to stimulate the enzyme[8–11]. The studies in the past have been mainly devoted to normal or cyclic animals, but hardly any study has been performed during pregnancy. The present study reports the effects of estradiol and progesterone on enzymes MAO and catechol-*O*-methyltransferase (COMT) during late pregnancy and parturition.

*Materials and methods.* White Sherman rats were used in all the study. The females were made pregnant as described previously and kept at 21 °C with natural night

and day cycles during April and May[12]. Parturition normally occurred between 21st and 22nd day of gestation. All the pregnant females received progesterone (4 mg/100 g), estradiol (0.6 mg/100 g) on 19, 20 and 21 days of gestation s.c. The treated females were killed by neck fracture at 0 h parturition after all the fetuses were born. The adrenals were excised and homogenized in 0.9% KCl at 0 °C for enzyme assays.

*Assay of COMT.* One adrenal was homogenized in 2 ml KCl and the homogenate was centrifuged for 30 min at 50,000 *g* at 1 °C. The incubation mixture consisted of 0.2 ml phosphate buffer (0.5 *M* at pH 7.9), 20 µl of Mg $Cl_2$, 20 µl of L-adrenaline[13], 0.2 ml of supernatant and 0.1 ml of S-adenosyl methionine-$^{14}$C-methyl (20 nmole). The mixture was incubated for 1 h at 37 °C. The reaction was stopped with addition of 0.5 ml of borate buffer (0.5 *M* at pH 10). The rest of the procedure was the same as described previously[13, 14].

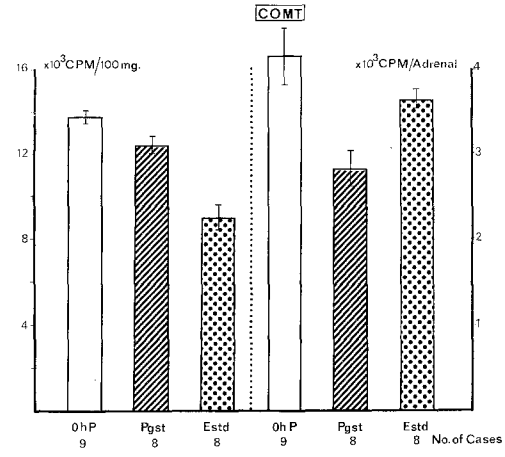


Fig. 1. Influence of exogenous administration of progesterone and estradiol to pregnant rats at 19, 20 and 21 days post coitum on catechol-*O*-methyltransferase activity (COMT) determined at 0 h parturition in the adrenal gland. Pgst, progesterone; Estd, estradiol; 0 h P, zero h after parturition.
Statistically the different groups differ:
0 h P and Pgst (cpm/adrenal gland) $p < 0.01$;
0 h P and Estd (cpm/100 mg) $p < 0.001$;
Pgst and Estd (cpm/adrenal gland and cpm/100 mg) $p < 0.001$.

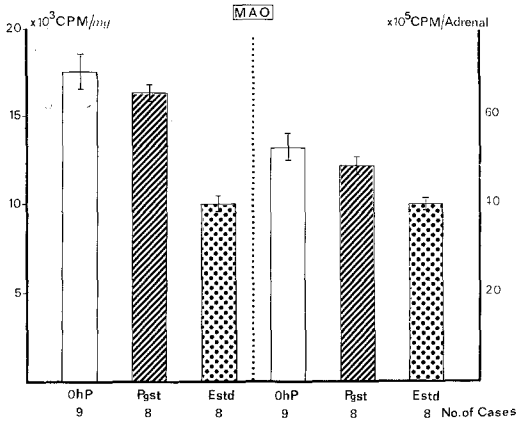


Fig. 2. Activity of enzyme monoamine oxidase (MAO) in the adrenal gland of pregnant rats subjected to progesterone and estradiol administrations on 19, 20 and 21 days post coitum. The activity of all the groups was determined at 0 h after parturition when all the fetuses were born. 0 h P, zero h after birth; Pgst, progesterone; Estd, estradiol. Significance of differences between the 3 groups are the following:
0 h P and Estd (cpm/mg) $p < 0.001$;
0 h P and Estd (cpm/adrenal gland) $p < 0.001$.
Progesterone administration did not induce any decrease of xtatistical significance.

*Assay of MAO.* The homogenate from enzyme COMT was further diluted 10 times with 0.9% KCl and 0.2 ml of it was used as enzyme preparation. The incubation mixture consisted of 0.25 ml of phosphate buffer (0.2 *M*, pH 7.4), 0.2 ml enzyme preparation and 0.1 ml of tryptamine $^{14}$C-bisuccinate (0.1 µCi). After 20 min incubation the reaction was stopped with addition of 0.2 ml 2 *N* HCl. The indole acetic acid formed was isolated in 4 ml acidified toluene as described before[14, 15].

*Results.* Figure 1 illustrates the effects of progesterone and estradiol administration to pregnant rats on activity of COMT in the adrenals determined at 0 h parturition. The activity declined significantly after progesterone injections from normal pregnant values. The decliine was more important when the results were expressed per adrenal gland. Estradiol resulted in highly marked decline in activity only when the results were expressed in cpm/100 mg of the gland. The activity after estradiol treatment was also lower (cpm/adrenal) but did not show any statistical significance.

Figure 2 shows the activity of enzyme monoamine oxidase in adrenal gland of female rats at 0 h parturition. The preadministration of progesterone on 19, 20 and 21 days post coitum did not show any marked modification in the enzyme monoamine oxidase activity in both cases of expressed results. The similar treatment with estradiol produced important declines in enzyme activity. The decrease after estradiol treatment was more marked when the results were calculated with cpm/mg of adrenal gland. However, in both ways of expressing the results, estradiol trratment produced declines of statistical significance.

*Discussion.* The results presented in this tudy show that exogenous administration of progesterone and estradiol during late pregnancy decrease monoamine metabolism by inhibiting enzymes monoamine oxidase and catechol-*O*-methyltransferase. The inhibitory effects

[1] The generous help of Dr. RAYNAUD of CEA, France is gratefully acknowledged. A.R.B. is pre-doctoral fellow of Paris University.
[2] R. J. WURTMAN, J. AXELROD and L. T. POTTER, J. Pharmac. exp. Ther. *144*, 150 (1964).
[3] R. J. WURTMAN, E. W. CHU and J. AXELROD, Nature, Lond. *198*, 547 (1963).
[4] G. G. S. COLLINS and J. SOUTHGATE, Biochem. J. *117*, 38 (1970).
[5] G. G. S. COLLINS, J. PRYSE-DAVIES, M. SANDLER and J. SOUTHGATE, Nature, Lond. *226*, 642 (1970).
[6] M. HOLZBAUER and M. B. H. YOUDIM, Br. J. Pharmac. *44*, 355 (1972).
[7] M. HOLZBAUER and M. B. H. YOUDIM, Br. J. Pharmac. *48*, 600 (1973).
[8] I. KAMBERI and Y. J. KOBAYASHI, J. Neurochem. *17*, 260 (1970).
[9] J. SOUTHGATE, G. G. S. COLLINS, W. POLLARD, J. PRYSE-DAVIES and M. SANDLER, J. clin. Path. *23*, suppl. *43* (1969).
[10] M. SANDLER, S. B. CARTER, B. L. GOODWIN, C. R. J. RUTHVEN, M. B. H. YOUDIM, E. Hanington, M. F. CUTHBERT and C. M. B. PARE, in *Neuropsychopharmacology of Monoamines* (Ed. E. USDIN, Raven Press, New York 1974), p. 3.
[11] R. J. WURTMAN and J. AXELROD, Biochem. Pharmac. *12*, 1417 (1963).
[12] S. PARVEZ, H. PARVEZ and D. GRIPOIS, Pharmac. Res. Commun. *5*, 265 (1973).
[13] R. J. WURTMAN and J. AXELROD, J. biol. Chem. *241*, 2301 (1966).
[14] H. PARVEZ and S. PARVEZ, Clin. chim. Acta *46*, 85 (1973).
[15] R. J. WURTMAN and J. AXELROD, Biochem. Pharmac. *12*, 1439 (1963).

of estradiol on activity of enzyme monoamine oxidase are in agreement with previous studies on normal and cyclic animals[16,17]. HOLZBAUER and YOUDIM[6,7] noticed increase in activity of enzyme monoamine oxidase after progesterone pretreatment of female rats during estrus cycle. But we find inhibition of the monoamine oxidase at 0 h parturition. This could be interpreted on the basis of 2 major discrepancies: 1. The endocrine status of pregnant rats during late gestation and at 0 h parturition. 2. Use of different substrates for monoamine oxidase assays.

The first point is the most important, since during late pregnancy there are marked fluctuations in the secretion of progesterone and estrogens in the rats. Estradiol concentration in plasma and ovaries increases several fold from 17th day post coitum to the onset of parturition[18] whereas progesterone content is greatly depressed during the last few days of gestation[19,20]. Moreover, the ratio of free and bound progesterone changes markedly approaching parturition[21]. This is why recent reports on the role of pregesterone in controlling monoamine metabolism in normal and cyclic animals cannot be compared with our present findings in pregnant rats due to great modifications in endocrine gland secretions during pregnancy. The second reason is the choice of substrate for enzyme assays. Recently it has been shown that both the enzymes of monoamine metabolism exist in many multiple forms, each having its own specific physicochemical properties. HOLZBAUER and YOUDIM[6,7] found that activity of monoamine oxidase was much higher when they used tyramine, kinuramine or dopamine as a substrate, while it was hardly changed using tryptamine as a substrate. We used tryptamine as a substrate for our monoamine oxidase assays. It may be an important explanation for the discrepancy in results. The role of endocrine function for regulation of monoamine metabolism during pregnancy can also be supported by our recent paper on the evolution of enzymes monoamine oxidase and catechol-*O*-methyltransferase during late pregnancy and parturition[22]. We observed variations of high statistical significance in release, storage and excretion of catecholamines during pregnancy in rats and rabbits[23,24].

From the present results, it is evident that estradiol and progesterone administered exogenously inhibit the processes of monoamine metabolism during late pregnancy. The mechanism of action of progesterone seems different in normal and pregnant animals, since endocrine activity is greatly modified due to gestation. Estradiol inhibition is stronger than progesterone. These results may have some clinical implication for toxaemic and irregular pregnancies.

[16] J. SOUTHGATE, in *Advances in Biochemical Pharmacology* (Eds. M. SANDLER and E. COSTA, Raven Press, New York 1972), vol. 5, p. 263.
[17] S. PARVEZ, D. Sci. Thesis Paris University 1975.
[18] A. A. SHAIKH, Biol. Reprod. *5*, 297 (1971).
[19] W. G. WIEST, W. R. KIDWELL and K. BALOUGH, Endocrinology *82*, 844 (1968).
[20] W. G. WIEST, Endocrinology *87*, 43 (1970).
[21] W. G. WIEST, in *Progesterone*, Ciba Foundation Study Group No. 34. (Eds. G. E. W. WOLSTENHOLME and J. KNIGHT; A. Churchill, London 1969).
[22] S. PARVEZ, H. S. PARVEZ and M. B. H. YOUDIM, Br. J. Pharmac. *53*, 241 (1975).
[23] S. PARVEZ, D. GRIPOIS and H. PARVEZ, Hormone Res. *5*, 245 (1974).
[24] S. PARVEZ and H. PARVEZ, Am. J. Obstet. Gynecol., in press (1975).

## Effets précoces de l'hormone de croissance sur les phosphates sériques et urinaires du rat

## Early Effects of Growth Hormone on Blood and Urinary Inorganic Phosphorus in the Rat

D. DURAND, M. PRÉLOT et Y. RAOUL

*Laboratoire de Physiologie, U.E.R. Mécanisme d'action des médicaments et des toxiques, 4, avenue de l'Observatoire, F–75270 Paris Cedex 06 (France), et Faculté mixte de Médecine et Pharmacie, F–49 Angers (France), 4 août 1975.*

*Summary.* From the kinetic study of the effects of one single growth hormone (GH) injection on the phosphate metabolism, it appears that the influence of GH on the serum phosphate level is biphasic: a decrease is followed by an increase. Conversely GH leads to an early decrease of the urinary phosphate excretion.

De nombreux faits expérimentaux et cliniques impliquent l'hormone de croissance (GH) dans le métabolisme de l'ion phosphate. Celle-ci fait disparaître l'hypophosphatémie consécutive à l'hypophysectomie[1]. Son hypersecrétion dans l'acromégalie[2] s'accompagne d'une hyperphosphatémie pouvant aussi être obtenue chez l'Homme[3] et chez l'animal à condition que l'hormone soit administrée pendant plusieurs jours[4] et qu'il y ait une imprégnation thyroxinique suffisante[5].

Cependant aucune étude des effets aigus de GH ne permet actuellement d'attribuer à GH un rôle dans l'homéostasie du métabolisme des phosphates et en particulier dans la régulation de la phosphatémie. Or cette régulation pose le problème de la composante hyperphosphatémiante. En effet la parathormone et la thyrocalcitonine, qui assurent la régulation de la calcémie par leurs effets inverses sur le métabolisme osseux, agissent dans le même sens sur le taux sérique des phosphates entraînant une hypophosphatémie. Hyperphosphatémiante à long terme, GH peut représenter le facteur tendant à élever la phosphatémie et à s'opposer ainsi aux effets hypophosphatémiants de la parathormone et de la thyrocalcitonine. Afin de vérifier cette hypothèse nous avons poursuivi l'étude cinétique des effets d'une injection unique de GH, donc à court terme, sur les phosphates sériques et urinaires du rat normal.

*Méthodes.* Les rattes Sprague Dawley utilisées, âgées de plus de 100 jours, reçoivent un régime standard (calcium; 0,81 g/100 g, phosphore 0.55 g/100 g) au

[1] C. H. LI, I. I. GESCHWIND et H. M. ÉVANS, Endocrinology *44*, 67 (1949).
[2] J. CORVILAIN et M. ABRAMOW, J. clin. Endocr. *34*, 452 (1972).
[3] H. GERSHBERG, J. clin. Endocr. Metab. *20*, 1107 (1960).
[4] M. PRÉLOT et E. LE BAS, C. r. Soc. Biol., Paris *161*, 1258 (1967).
[5] D. DURAND et M. PRÉLOT, à paraître (1975).