3 Goodman, R.L., Hotckiss, J., Karsch, F.J., and Knobil, E., Biol. Reprod. *11* (1974) 624.
4 Perachio, A.A., Alexander, M., Murr, L.D., and Collins, D.C., Steroids *29* (1977) 21.
5 Nieschlag, E., and Wickings, E.J., in: Non-human primate models for study of human reproduction, p.136. Ed. T.C. Anand Kumar. Karger, Basel 1980.
6 Michael, R.P., Setchell, K.D.R., and Plant, T.M., J. Endocr. *63* (1974) 325.
7 Rose, T.M., Gordon, T.P., and Bernstien, I.S., J. Endocr. *76* (1978) 67.
8 Puri, C.P., Puri, V., and Anand Kumar, T.C., J. med. Primatol. *9* (1980) 39.
9 Anand Kumar, T.C., David, G.F.X., Sharma, D.N., Puri, C.P., Puri, V., Dubey, A.K., Sehgal, A., Sankaranarayanan, A., and Pruthi, J.S., in: Non-human primate models for study of human reproduction, p.37. Ed. T.C. Anand Kumar. Karger, Basel 1980.
10 Puri, C.P., Puri, V., David, G.F.X., and Anand Kumar, T.C., Brain Res. *200* (1980) 377.
11 Hall, P.E., The World Health Organization's Programme of the standardization and quality control of radioimmunoassay of hormones in reproductive physiology. Horm. Res. *9* (1978).
12 WHO method manual. Programme for the provision of matched assay. Reagents for the radioimmunoassay of hormones in reproductive physiology, 3rd edn, 1979. WHO Special Programme of Research in Human Reproduction. World Health Organization, Geneva, Switzerland.
13 Snedecor, G.W., and Cochran, W.G., Statistical methods, 5th edn. The IOWA State University Press, 1961.
14 Perlow, M.J., Repperl, S.M., Boyar, R.M., and Klein, D.C., Neuroendocrinology *32* (1981) 193.
15 Mukku, V., Prahalada, S., and Moudgal, N.R., Nature *260* (1976) 778.

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Effect of oestradiol on RNA polymerase of foetal guinea-pig uterus

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Summary. The administration of 10 µg oestradiol to the foetus of the guinea-pig (55-65 days of gestation) causes an increase in RNA polymerase I and II activities in the nuclei of the foetal uterus. RNA polymerase I activity increased by 4 times above the control values after 120 min ($p < 0.001$) whereas RNA polymerase II activity increased more rapidly, reaching 2.5 times the control levels at 30 min and 4 times by 120 min after treatment ($p < 0.01$).

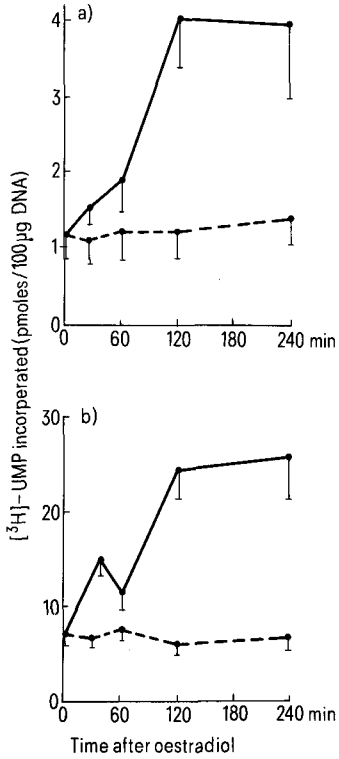
In previous studies, it has been established that administration of oestradiol increases uterine wet weight[1] and provokes a stimulation of progesterone receptors[2] in the foetal uterus of the guinea-pig. Futhermore, these 2 parameters are correlated with the translocation and nuclear retention of oestradiol receptors[3]. It was also demonstrated that the foetal uterus responds to oestradiol by an intensification of nucleosomal histone acetylation[4] as well as by an increase in the incorporation of ^{3}H-leucine into acid-insoluble proteins[5]. Since several oestrogenic effects appear to be correlated with the increase in RNA polymerase activities during extrauterine life[6,7], it was interesting to investigate the effect of oestradiol on the RNA polymerase activities in the foetal uterus.

Hartley albino guinea-pigs of 55-65 days of gestation were purchased from a commercial breeder (Hiblot, Vigneux-sous-Montmedy, Meuse, France). Animals were anesthetized with 5% (w/v) pentobarbital - 0.006% (w/v) atropine and submitted to laparotomy. Each female foetus was injected s.c., in situ, with 10 µg of oestradiol in 10% ethanol-saline solution. The controls received vehicle alone. The foetuses were replaced in the abdomen of the mother and at different times (30, 60, 120, 240 min) removed and decapitated. The foetal uteri were then excised and stripped of fat. Tissues were homogenized in 3-4 ml of 0.25 M sucrose in TKM buffer (0.25 M sucrose - 0.05 M Tris-HCl - 0.025 M KCl - 0.005 M $MgCl_2$, pH 7.4) and centrifuged at 900×g for 10 min. The 900×g pellet was washed with 0.4 M sucrose in TKM buffer (1-2 ml) and centrifuged at 900×g. Finally, this pellet was homogenized in 2 M sucrose in TKM buffer (6 ml), layered on an equal volume of the same solution and centrifuged at 250,000×g for 60 min to obtain the purified nuclei.

The pellet containing the purified nuclei was resuspended in a small volume (1-2 ml) of TEDG buffer (0.05 M Tris-HCl - 0.0001 M EDTA - 0.0005 M dithiothreitol - 25% (v/v) glycerol, pH 7.9) for measurement of endogenous RNA polymerase activities. These activities were evaluated essentially by incubation at 37 °C for 30 min of an aliquot (0.05 ml) of the nuclear suspension with a same volume of reaction mixture containing 0.075 µmoles of ATP, CTP, GTP and 1 µCi (0.0005 µmoles) of ^{3}H-UTP (sp.act. 2 Ci/mmole, Amersham, Versailles, France) in either low ionic strength medium in the presence of Mg^{2+} (0.2 µmoles of $MgCl_2$) plus α-amanitin (1µg) for the evaluation of the RNA polymerase I activity, or high ionic strength medium in the presence of Mn^{2+} (0.2 µmoles of $MnCl_2$) for the measurement of RNA polymerase II activity, according to the method described by Borthwick and Smellie[8]. The enzymatic reaction was stopped by immersing the samples in crushed ice and an aliquot of each incubation was spotted on filter paper discs (Whatman GF/A 2.5 cm) and washed as described[8] with different solvents (10 and 5% (w/v) trichloracetic acid containing 1% (w/v) tetrasodium pyrophosphate, alcohol, alcohol-ether, ether). The discs were placed directly in a counting vial and, after drying, were treated for several hours with 0.5 ml of Soluene 350 (Packard Instruments S.A., Rungis, France). The incorporated radioactivity was measured in 3 ml scintillation fluid (PPO 0.5%-POPOP 0.01% (w/v) in toluene). Protein was measured according to the method of Lowry et al.[9] and the DNA determinations were carried out by the method of Burton[10].

Figure a shows that within the 1st hour after administration of 10 µg oestradiol to the foetus, the RNA polymerase I activity was not significantly different from control values, but by 120 min it was 4 times higher. Thereafter, the activity remained constant between 120 and 240 min. Figure b shows that the RNA polymerase II activity increased rapidly to reach 2.5 times the control values after

30 min; there was a 4-fold increase at 120 min. The level remained constant till 240 min. In contrast, in the foetal kidney, after 120 min of oestradiol treatment, no increase was observed. The control values for RNA polymerase activities I and II were 0.7 pmoles/100 µg DNA ± 0.24 (SEM) and 3.8 ± 0.6 (SEM) respectively, and in oestradiol treated foetuses were 0.8 ± 0.6 (SEM) and 4 ± 0.36 (SEM).



Effect of oestradiol treatment on RNA polymerase I and II activities in the uterine nuclei of foetal guinea-pig. Foetuses were removed at different times up to 240 min after a single injection of oestradiol as described in the text. Uterine nuclei were prepared and endogenous RNA polymerase activities determined. *a* RNA polymerase I activity: oestrogen treated (—) and control (- - -). *b* RNA polymerase II activity: oestrogen treated (—) and control (- - -) uteri. All points represent the mean ± SEM from 3-4 determinations.

This is an additional effect to the different oestrogenic responses already observed in the foetal uterus and, to our knowledge, has not been described in the foetus. It is interesting to note that the increase in RNA polymerase activities above control values within the 1st 240 min of treatment is similar to that of immature or ovariectomized rabbit and rat[8,11].

Since the foetal uterus of the guinea-pig responds to oestradiol by a very rapid increase in histone acetylation[4] (after 10 min of treatment), it is possible that this enhancement of histone acetylation might be the first step before the increase in RNA polymerase activities. The latter might precede the incorporation of ^{3}H-leucine into acid-insoluble proteins previously observed[5]. Moreover, it has been demonstrated that oestradiol treatment provokes the induction of a specific protein, namely the progesterone receptor[2,12]. Consequently, it can be suggested that in this foetal tissue there exists a correlation between the activation of RNA polymerases by oestradiol and the synthesis of proteins.

1 Pasqualini, J.R., and Nguyen, B.L., Experientia *35* (1979) 1116.
2 Pasqualini, J.R., and Nguyen, B.L., Endocrinology *106* (1980) 1160.
3 Sumida, C., and Pasqualini, J.R., J. Receptor Res. *1* (1980) 439.
4 Pasqualini, J.R., Cosquer-Clavreul, C., Vidali, G., and Allfrey, V.G., Biol. Reprod. *25* (1981) 1035.
5 Sumida, C., and Pasqualini, J.R., Experientia *37* (1981) 782.
6 Hardin, J.W., Clark, J.H., Glasser, S.R.G., and Peck, Jr, E.J., Biochemistry *15* (1976) 1370.
7 Anderson, J.N., Peck, Jr, E.J., and Clark, J.H., Endocrinology *96* (1975) 160.
8 Borthwick, N.M., and Smellie, R.M.S., Biochem. J. *147* (1975) 91.
9 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. biol. Chem. *193* (1951) 265.
10 Burton, K., Biochem. J. *62* (1956) 315.
11 Glasser, S.R., Chytil, F., and Spelsberg, T.C., Biochem. J. *130* (1972) 947.
12 Pasqualini, J.R., and Nguyen, B.L., J. Endocr. *81* (1979) 144 P.

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Pancreatic islet cell suspensions from newborn rats; different preparation procedures, viability and (pro)insulin biosynthesis[1]

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Summary. Islet cell suspensions were prepared from neonatal rat pancreatic islets. While mechanical disintegration results in a higher yield, cells prepared by trypsin treatment appear to be better preserved. Trypsin treatment of pancreatic islets during the cell preparation procedure does not influence the stimulation by glucose of (pro)insulin biosynthesis in freshly isolated cells.

The isolation of pancreatic islet cells of different species[2-8] has become an important tool for the investigation of special problems in experimental diabetes research.

Cell suspensions have been prepared from isolated islets of Langerhans by mechanical disintegration[4], enzymatic treatment[2,3,6,9] or a combination of both methods[5]. We compared 2 of these procedures with respect to the yield and the stability of the cells. Because the glucose stimulation of (pro)insulin biosynthesis of cells prepared by mechanical disintegration has already been shown[10,11], the question