plasma can be isolated and electrophoretically identified with some changes in arginine-rich H3-H4 fractions, and this confirmed our previous study[4].

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Progesterone receptors in the foetal uterus of guinea-pig: Its stimulation after oestradiol treatment

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Summary. This paper shows for the first time the presence of progesterone receptors in the foetal guinea-pig uterus, as well as the stimulation of progesterone receptors in foetal uterus in animals treated with oestradiol.

Previous studies in this laboratory have demonstrated the presence of oestradiol receptors in the foetal uterus of guinea-pig[1]. These receptors appear in this foetal tissue at an early age of gestation, increase during foetal development and decrease after birth[2]. In this paper, the existence of progesterone receptors in the same foetal tissue, at the end of gestation, and its stimulation in oestradiol-treated animals are presented.

Foetuses of Hartley Albino guinea-pigs (50 days to the end of gestation) were used. Females and males were mated for 24 h, consequently the days of gestation were established with an error of ±24 h. Experiments were carried out after incubation of ^{3}H-progesterone (s.a.: 55 Ci/mmole, NEN Chemical, GmbH, Frankfurt, GFR), with the total cell (cell suspensions) or with the cytosol fraction in Krebs Henseleit buffer. The subcellular fractionation of the foetal uterus was carried out according to the method of Chauveau et al.[3]. The cytosol fraction was obtained by centrifugation at 250,000× g. Purified nuclei were extracted successively with the following solutions a) 0.1 M TRIS.HCl - 0.0015 M EDTA (0.1 M TRIS); b) 0.3 M NaCl - 0.01 TRIS.HCl (0.3 M NaCl) and c) 1 M NaCl - 0.01 M TRIS.HCl (1 M NaCl). Sucrose density gradients were carried out in 5-20% w/v sucrose solutions containing 0.012 M thioglycerol. Centrifugations were carried out at 250,000× g at 2 °C for 16 h. Proteins were measured according to the method of Lowry et al.[4]. Specific binding was determined after incubation of the total cell or the cytosol fraction with ^{3}H-progesterone alone or with a 100-fold excess of unlabelled progesterone, and calculated by the difference in binding between these 2 incubations as determined using the dextran charcoal method[5]. Equilibrium constants were measured by the Scatchard method[6].

Figure 1 shows an example of a Scatchard plot for ^{3}H-progesterone binding in the cytosol fraction of the fetal guinea-pig uterus. The dissociation constant is ($Kd_{4°}$) 3.6 ± 10^{-9} M and the number of sites $n = 180 \pm 28 \times 10^{-15}$ moles/mg protein. Progesterone and the synthetic progestagen R-5020 (17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione) compete significantly in the formation of the ^{3}H-progesterone-protein complex, but oestradiol, oestrone or cortisol have no effect. The synthetic progestagen ^{3}H-R-5020 was also found to bind specifically in the cytosol fraction of the uterus.

The analysis by ultracentrifugation in sucrose gradient shows the presence of a component with a sedimentation coeficient of 6-7 S and another with 4-4.5 S. It is interesting to note that similar components were found for the ^{3}H-progesterone complex in human endometrium[7]. The specific protein which binds progesterone in the foetal uterus is not a contamination of foetal plasma protein, which also binds progesterone[8], because in the cytosol fraction of the foetal uterus which was heated for 1 h at 37 °C before being incubated with ^{3}H-progesterone (4×10^{-9} M) at 4 °C for 4 h, it was observed that 85-95% of the specific binding sites of progesterone were destroyed. No effect of temperature was observed in the formation of the ^{3}H-progesterone protein

Table 1. Effect of oestradiol on the weight of foetal uterus of guinea-pig after injection of oestradiol to the mother

Days of gestation	Control animals (in mg per uterus)	Treated animals
56-57	40.0±3	67.0±7.0
58-59	41.5±4.5	80.7±6.6
64-65	93 ±3	167 ±13.1

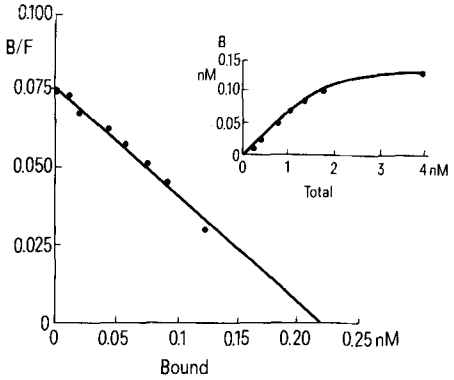
Each pregnant guinea-pig received 1 mg/kg/day of oestradiol. The values correspond to the data obtained in 4 uteri of 3 experiments (means ± SD).

Table 2. Effect of oestradiol on the quantity of progesterone receptors in the foetal guinea-pig uterus

Days of gestation	Subcellular fraction	Specific progesterone binding sites (fmoles/mg protein) Controls	Treated animals
48-52[a]	Cytosol	29	97
60-65[a]	Cytosol	66	407
58-59[b]	Cytosol	45	175
	Nuclear extracts		
	0.1 M Tris	ND	15
	0.3 M NaCl	ND	12
	1 M NaCl	3	48

Pregnant guinea-pigs were treated as indicated in Table 1.
[a] Incubation of the foetal cytosol fraction with ^{3}H-progesterone (4×10^{-9} M) with or without a 100-fold excess of unlabelled progesterone 4 h at 4 °C. [b] Incubation of cell suspensions with ^{3}H-progesterone (8×10^{-9} M) with or without a 100-fold excess of unlabelled progesterone 15 min at 37 °C. The data represent the average values with 4 uteri of 3 experiments.
ND, not detectable.

complex of the foetal plasma processed in the same experimental conditions. The number of specific progesterone binding sites after incubation of ^{3}H-progesterone (4×10^{-9} M) with the cytosol fraction of the foetal uterus is 29.2±2.9 fmoles/mg protein at 48–52 days of gestation and increases to 66.2±3.8 at 60–65 days of gestation (4 experiments). At this period of gestation, the number of oestradiol receptors in the foetal uterus of guinea-pig is very high[2], which suggested that oestradiol could stimulate the progesterone receptors in the foetal uterus at the end of gestation. To demonstrate this, we first observed that tritiated oestradiol injected to the mother could cross the placenta and reach the foetus (0.20–0.30% of the injected radioactivity was found in each foetus, 30 min after s.c. injection of 50 µCi of ^{3}H-oestradiol to the mother, from which 10–15% of the total radioactivity in the foetus was found as unmetabolized oestradiol). Following this, 1 mg/kg/day of oestradiol was injected to pregnant guinea-pigs for 3 days and the quantity of specific progesterone binding sites were evaluated in the treated and in the control animals. Table 1 indicates the effect on the weight of the foetal uterus and table 2 indicates the quantitative values of ^{3}H-progesterone receptors. As is shown, there is a significant increase in the weight of the foetal uterus and the number of progesterone receptors increases 4–7 times. It is concluded that the response effect of oestradiol on the production of uterine progesterone receptors is present during foetal life.



Scatchard plot of the ^{3}H-progesterone specific binding in the cytosol fraction of the foetal uterus of guinea-pig. Uterine cytosols of the foetal guinea-pig (55–64 days of gestation) containing 1.5 mg of protein (ml) were incubated with various concentrations of ^{3}H-progesterone ($1.65–40.0\times10^{-10}$ M) in the absence or presence of 4×10^{-7} M of unlabelled progesterone for 4 h at 4 °C. Specific binding was calculated using the charcoal-dextran method.

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Lack of specific neurons in the ventral nerve cord for the control of prothoracic glands[1]

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Summary. There is a distinct serial homology in the distribution of afferent neurons in different ventral ganglia of wax moth larvae. No neurons specific to the thoracic ganglia which innervate the prothoracic glands were found. 2 peptidergic cells in the suboesophageal and 4 such cells in the prothoracic ganglion probably send axons to the glands, but seem to release their secretion also at other sites. Specific control of prothoracic glands is possible providing that liberation of neurohormones is regulated at the axonal level.

Function of the prothoracic glands (PTG) of the larvae of *Galleria mellonella* L. is stimulated by prothoracicotropic neurohormone from the brain and inhibited by unknown factor(s) from the prothoracic and mesothoracic ganglia[3,4]. It is considered that under physiological conditions this factor is transported to PTG via the peptidergic neuraxons which terminate within the glands[5–7]. The glands are innervated from the suboesophageal (SG), prothoracic (T_1) and mesothoracic (T_2) ganglia by nerves which branch to dorsal body wall musculature, stigmata, tracheae, gut, fat body, and apparently other viscera (figure 1). In an effort to find neurons innervating specifically the PTG, we compared locations of central perikarya of these nerves[8] with those of homologous nerves in the metathoracic (T_3) and several abdominal ganglia (A_1, A_4, A_5) which do not innervate PTG.

Material and methods. The neurons were visualized by means of cobalt filling technique[9,10]. Ganglia dissected from fully grown larvae were placed in cold Grace's medium or *Galleria* saline (20 mM NaCl, 30 mM KCl, 15 mM $MgCl_2$, 8 mM $CaCl_2$, 5 mM $NaHCO_3$, 5 mM KH_2PO_4, 220 mM sucrose) and the nerve to be filled was dipped across a fine oil barrier into 0.3 M $CoCl_2$ in saline. After 14–24 h incubation, the preparation was washed with saline and immersed for 10–30 min in 1% glutaraldehyde solution made by dilution of 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2) with the saline to which was added 2% sucrose. Preparation was then treated with 5–10% $(NH_4)_2S$ in saline, fixed in 3% glutaraldehyde for 2 h, transferred in cacodylate buffer to room temperature, dehydrated through ethanol solutions (30, 50 and 70% solutions were adjusted to pH 7.2 with phosphate buffer (0.1 M) and 90 and 96% solutions with solid TRIS) and propylene oxide, and embedded in Durcupan. No detectable loss of CoS occurred in blocks stored for several months. Preservation of the tissue was sufficient also for EM studies.

Results and discussion. The cervical nerve of SG (figure 1) contains axons of 3 ventromedial neurons (group 1 in figure 2) and centripetal axons terminating ipsilaterally in SG. No connection between right and left cervical nerves