Corticosteroid synthesis by the adrenal glands of 22-day-old fetuses incubated with 4-^{14}C progesterone and dexamethasone for 1 h (dpm/mg)

Steroids	Controls	Dexamethasone µg/ml incubation medium 0.1	0.2	0.5	1.0	2.0
Aldosterone	47.4[a] ±5.7	36.0 ±1.0	31.6 ±0.4	18.5[b] ±1.4	17.1[b] ±5.1	9.6[b] ±0.1
18-Hydroxy-11-deoxycorticosterone	403.7 ±15.3	424.8 ±2.7	428.5 ±6.5	466.1 ±17.9	503.0 ±15.0	544.0[c] ±33.0
Corticosterone	334.1 ±8.9	273.7 ±20.5	264.4 ±5.0	261.2[c] ±8.8	233.7[b] ±11.8	231.0[b] ±9.8
11-Deoxycorticosterone	196.0 ±21.0	224.4 ±12.2	269.9 ±37.1	291.5 ±1.4	236.0 ±17.0	233.0 ±45.0
Progesterone (residual substrate)	21,902.1 ±982.1	22,025.5 ±376.0	21,891.2 ±899.4	22,742.1 ±114.4	22,202.3 ±237.8	21,081.8 ±498.3

Values are mean ± S. E. [a] 4 fetuses per sample; [b] $p < 0.01$ (vs control); [c] $p < 0.05$ (vs control).

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procedural losses from the recovery at the last stage of purification of non-radioactive standards added immediately after incubation. The results obtained were statistically evaluated by the analysis of variance. In the case of homogeneity of variances, Student's *t*-test was used, and Kramer's test when the variances were not homogeneous.

Results and discussion. The results indicate that fetal adrenal glands in vitro hidroxylated 4-^{14}C progesterone in 11-deoxycorticosterone, 18-hydroxy-11-deoxycorticosterone (18-OH-DOC) corticosterone and aldosterone. The major steroid produced was 18-OH-DOC. The adrenal glands of fetuses incubated with 0.5–2.0 µg dexamethasone/ml incubation medium synthetized less corticosterone and aldosterone than the adrenal glands of control fetuses. However, with higher concentrations of dexamethasone the conversion of 4-^{14}C progesterone to 18-OH-DOC was increased.

It is known that dexamethasone inhibits the fetal adrenals by decreasing fetal ACTH activity[12,13]. This experiment demonstrated in vitro inhibition of 11β-hydroxylation of progesterone to corticosterone by the high concentrations of dexamethasone. Therefore the results indicate that dexamethasone may affect steroidogenesis in the fetal adrenal gland directly, as well as its already known inhibitory effect mediated by the fetal pituitary[12,13].

RNA Biosynthesis in Isolated Prothoracic Glands of *Tenebrio molitor* in vitro

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Summary. The prothoracic Glands of *Tenebrio molitor* synthesize in vitro mainly rRNA. The rate of RNA synthesis reaches a maximum at day 10 of the last larval instar, which coincides with the event of apolysis.

The prothoracic glands (PGs), the sites of biosynthesis of the moulting hormone α-ecdysone[2–4] in insects, undergo periodic morphological changes during growth and metamorphosis which can be correlated with their secretory activities. Prior to each secretory activity, an increase in nuclear and nucleolar volume is observed[5] which is followed by high cytoplasmic vacuolization, abundant appearance of rough endoplasmic reticulum and Golgi areas[6]. The activation is brought about by neurohormones from the brain[7]; this stimulation can be demonstrated by an increased ^{3}H-uridine incorporation into RNA[8,9], which was also demonstrated after addition of brain hormone extracts to prothoracic glands in vitro[10]. Since the activated glands retain their ability to synthesize α-ecdysone both after transplantation[11] and in vitro[2–4], it seemed reasonable to assume that they would also be able to maintain their RNA biosynthetic

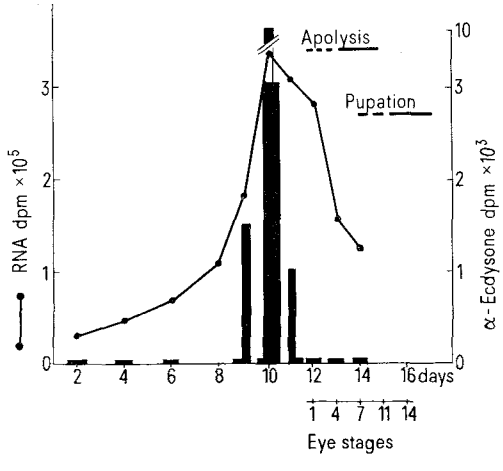

Fig. 1. RNA and α-ecdysone biosynthesis of PGs in vitro: total RNA synthesis of 10 gland pairs in 24 h (– ● – ● –), mean values of 2 independant series; α-ecdysone synthesis: each bar represents a single incubation. Eye stages are given according to [14].

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activity under in vitro conditions. We therefore investigated the ability of prothoracic glands from last larval instars of *Tenebrio molitor* to synthesize ribonucleic acids in concomitance with their α-ecdysone synthesizing capacities.

Material and methods. Mealworms were bought from local suppliers and kept at 25 or 27 °C on a 1:1 mixture of wheat bran and shredded soya-beans supplemented with fresh carrots. Twice a day the freshly moulted larvae were collected. Only those weighing 80–100 mg were used in the experiments. The PGs were prepared and cultured in vitro as described by ROMER et al.[4]. 5 μCi of 5.6-^{3}H-uridine (spec. act. 45 Ci/mmole; from NEN, Dreieichenhain) were added to 600 μl of culture medium in which 5 pairs of PGs were cultured for 24 h.

RNA was isolated by the SDS-pronase method described by EDSTRÖM and TANGUAY[12] and separated by polyacrylamide gel electrophoresis (2.5% gels in 0.04 *M* *Tris*-Na^+-acetate buffer pH 7.2) for 90 min at 5 mA constant current. The gels were cut into 2 mm sections with a Gilson gel fractionator, dissolved in 100 μl 30% H_2O_2 and counted in 5 ml Aquasol (NEN, Dreieichenhain) in a Berthold Liquid Scintillation Counter with an efficiency of 28–31%. The radioactivity in the RNA peaks of each gel was integrated (see Figure 1). Reference gels were fixed with acetic acid and stained with methylene blue[13].

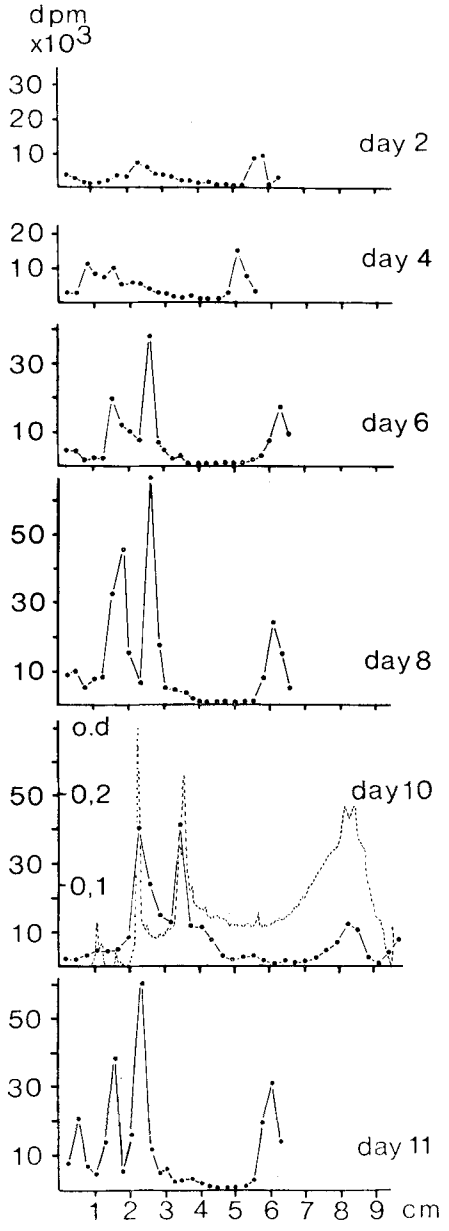

Fig. 2. ^{3}H-RNA profiles in polyacrylamide gels of PGs of different ages cultured for 24 h in vitro. Radioactivity (. – . – .); OD_{560} of methylene blue stained carrier RNA from *Tenebrio* fat body (– – –).

α-Ecdysone biosynthesis was checked in parallel cultures containing 5 μCi 1,2-^{3}H cholesterol (spec. act. 52 Ci/mmole, NEN, Dreieichenhain) dissolved in 0.75 μl Tween 80. 10 pairs of PGs were incubated for 48 h. Isolation of the moulting hormone containing fraction was performed as described[4].

Results and discussion. Both the number of larval instars and the duration of the last larval instar of *Tenebrio molitor* vary considerably[14], but larvae weighing 80–100 mg immediately after the larval molt are in the last instar with a probability of 97%. For more than 60% of these animals, the duration of this instar is 16 days. The timing from the date of the last larval molt is less precise than the classification into 'eye stages' according to STELLWAAG-KITTLER[14], but this method only covers the last 5 days of the instar. The RNA biosynthetic activity, however, increases much earlier, reaching a maximum between day 10 and 12 (Figure 1), which coincides with the time of apolysis.

The acrylamide gel profiles (Figure 2) show that ribosomal RNA is preferentially synthesized during these days. This coincides with the observation that rough endoplasmic reticulum becomes more abundant during this stage[6]. Also BRÄUER et al.[15] report a α-amanitin insensitive rRNA synthesis in activated PGs of *Periplaneta*. It might be that an intense protein biosynthesis, e.g. of cholesterol transforming enzymes, is a prerequisite for the subsequent production of α-ecdysone. Unfortunately the culture of PGs in vitro was unsuccessful during the last 2 days of the instar. The larval tissues begin to histolyze during the stage and the excised PGs are then unavoidably contaminated with the fat body tissue. They quickly lose their structural integrity in the tissue culture and do not produce any more appreciable amounts of RNA or α-ecdysone.

Concomitantly with the peak of RNA synthesis, the glands produce some labelled α-ecdysone from ^{3}H-cholesterol during day 9–11 (eye stage 1). The amount of labelled moulting hormone is, however, relatively small and does not exceed 40 pg α-ecdysone per 10 pairs of PGs (calculated from the specific activity of the ^{3}H-cholesterol). This may be due to a sufficiently large pool of unlabelled cholesterol in the glands[16] or another precursor of the ecdysone biosynthesis. Also, DELBÈCQUE et al.[17] found only small amounts of moulting hormones in total homogenates of *Tenebrio* larvae of this stage. They report 2 peaks of β-ecdysone titers at day 13 and 14 (eye stages 8–13). It remains open whether this high concentration of moulting hormone originates in vivo from the PGs or from other tissues[15, 18].

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