Effects of neonatal administration of sex steroids on development of vagina in female rats

Groups and treatment		Age (days)	No. of rats in group	No. of rats with vaginal orifice	Age in days at visible vaginal opening (mean ± SD)
Ia	Intact control		17	17 (100.0%)	43.3 ± 4.2
IIa	E_2	0	16	16 (100.0%)	9.1 ± 2.0
IIIa	TP	0	28	0 (0%)[a]	-
b	TP	1	12	0 (0%)[a]	-
c	TP	2	16	2 (12.5%)[a]	36,40
d	TP	3	17	17 (100.0%)	12.9 ± 3.8[a]
e	TP	5	26	26 (100.0%)	18.7 ± 9.8[a]

E_2, 17β-estradiol (10 µg), TP, testosterone propionate (1.25 mg). [a] Differs from I; $p < 0.001$.

produce keratinization, it is reasonable to presume that the cytodifferentiation concerning the converting system of TP to E_2 might be established in vaginal epithelium when females are 3 days of age. The results obtained in this investigation by histochemical methods also show that the stromal cells around the vagina in 0-day rats are capable of responding to TP. On the basis of the general principle that mesenchyme largely controls morphogenesis[12,13] and the finding of an other study on mammary morphogenesis, showing that testosterone-activated mesenchymal cells condense mammary gland epithelium and cause the eventual destruction of mammary epithelium[14], it may be that TP-activated stromal cells around the vagina play an important role in the induction of the atresia of the vagina. However, the detailed process is not clarified. Thus, further studies are necessary.

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Effect of the photoperiod on corpus allatum activity in vitro in the beetle, *Pterostichus nigrita* F.

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Summary. In the carabid beetle *Pterostichus nigrita* reproduction is controlled by photoperiods and the corpus allatum hormone. Corpora allata were incubated in vitro and the release of juvenile hormone was quantified. Short-day conditions induced low activity of the corpora allata; long days, after short-day treatment, stimulate high corpus allatum activity, while long days alone have no effect.

In the carabid beetle *Pterostichus nigrita* sexual maturation is controlled by short-day (SD) and long-day (LD) photoperiods[1,2]. If female beetles are placed in LD conditions immediately after eclosion, the ovaries remain pupal. If the beetles are placed in SD, however, their ovaries develop previtellogenic oocytes but no yolk deposition can be observed. SD periods with less than 15 h of light induce such previtellogenesis (PVG) in more than 50% of the individuals. This stage of PVG can be overcome, when the female beetles are transfered from SD photoperiods into LD. Now, the 2nd step of maturation, vitellogenesis (VG) begins, the oocytes start to grow and the eggs are oviposited. Days with more than 13 h of light induce VG in more than 50% of the SD females. In this way the changing photoperiods from autumn until spring result in an exact timing of reproduction in springtime in Central Europe[2]. However, in this species the photoperiodic reaction is flexible and varies with geographical latitude as demonstrated for a subarctic population[3]. The species used for the reported experiments belongs to the β-form of the 2 biospecies known for *P. nigrita*[4].

The present experiments were designed to analyze the relationship between photoperiodic control and corpus allatum (CA) activity during maturation in *P. nigrita*. In a number of recent experiments, the hormonal basis of this 2-step photoperiodically controlled reproduction has been analyzed[2]. In view of these results the following hypothetical model was tested: Short days bring about low activity of the CA resulting in a low titer of JH in the hemolymph. As a result of this weak hormonal stimulation PVG occurs. Long days following the short-day treatment increase the JH secretion of the CA and eggs are produced. Long days alone do not stimulate the endocrine system.

To demonstrate this 2-step CA-activity, excised glands (CA

with attached corpora cardiaca) of immature, partly mature and fully mature beetles were incubated in vitro for 4 h with ^{14}C-methyl-methionine as recently described by Tobe and Pratt[5]. After extraction with ethyl acetate, the incubation medium was subjected to TLC and the spots containing JH were assayed using a liquid scintillation counter. In addition, the dry weights of the ovaries of the experimental females were determined.

CA of immature females maintained in LD exclusively synthesize very small amounts of JH (fig. 1). Previtellogenic, SD females secrete substantial amounts of JH. The SD effect seems to be time-dependent; 9-week-old females produce 25% more JH than 6-week-old ones (0.775 pM h^{-1} $pair^{-1}$ and 0.575 pM h^{-1} $pair^{-1}$ respectively). After the change from SD to LD, JH synthesis increases dramatically. Until day 10 after the change of photoperiodic regimen synthesis increases by a factor of 2.6, and it remains high for at least a further 3 weeks (up to 1.775 pM h^{-1} $pair^{-1}$). The type of JH synthesized is as yet unknown.

The change in the activity of the CA corresponds with the ovarian dry weight of the females from which the incubated CA were taken (fig. 2). Although previtellogenic ovaries have enlarged terminal oocytes, they do not differ in weight from undeveloped ovaries. Mature ovaries increase in weight because of yolk deposition.

Maturation in male *P. nigrita* is also controlled by photoperiods[6]. Usually, they remain immature in LD photoperiods but they reach complete sexual maturity when maintained in SD without subsequent transfer into LD. Copulation of mature SD males with previtellogenic SD females was observed and sperms were found in the seminal receptacles of these females. Implantation of active SD CA (male or female donor) or injection of JH brings about complete maturation in undeveloped LD males[7]. As in females, implanted CC have no effect[2,7]; in vitro incubation of male CA had the results shown in figure 3; CA of males maintained in LD are more or less inactive; the CA of SD males (age 6 weeks) produce 0.282 pM h^{-1} $pair^{-1}$ JH, those of short-day beetles aged 9 weeks 0.476 pM h^{-1} $pair^{-1}$ JH. Surprisingly, the activity of the male CA increases after the beetles have been transferred from SD into LD (up to 1 pM h^{-1} $pair^{-1}$ JH at day 30). In general the CA of these males produced only about half as much JH as those of females treated in the same way.

Many insect species require JH for maturation. Whereas high JH titers are necessary for complete oogenesis in females of *P. nigrita*, males mature and copulate in the presence of rather low JH titers. These results are in agreement with recent CA implantation experiments[2,7]. Thus, the significance of the increase of the JH titer in male beetles after transfer from SD into LD remains unclear. Possibly, the increased JH titer improves maturation and influences sexual behaviour[8]. The changes of the JH titers in SD females or in females after exposure to LD following SD, however, can clearly be correlated with previtellogenic and vitellogenic phases of oogenesis. The size of the CA reflects their activity in *P. nigrita*. A possible effect of

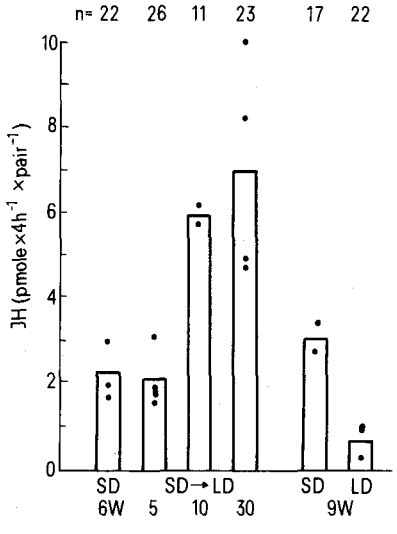

Figure 1. JH synthesis in vitro by female corpus allatum (CA) of *Pterostichus nigrita* after exposure to constant short-day (SD) or long-day (LD) for 6 or 9 weeks (W) and after transfer from SD (6 weeks) into LD at day 5, 10 and 30. Each incubated group (●) consisted of at least 4 CA with attached CC; (n), total number of incubated CA.

CA were incubated in MEM medium (Serva, Heidelberg) with Hank's salts and L-glutamine and without sodium bicarbonate, containing 20 mM Hepes and fortified with 20 mg/ml Ficoll 400 (Serva). L-(methyl-^{14}C)-methionine was incorporated into the medium at a final sp. act. of 40.4 mCi/mM.

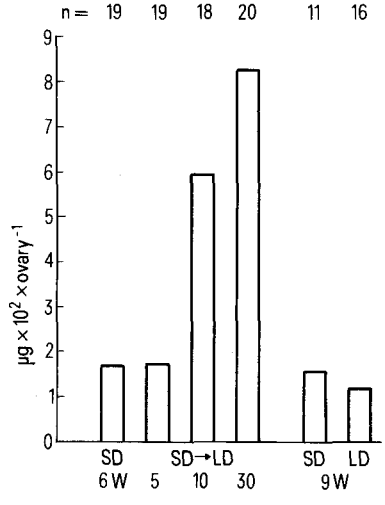

Figure 2. Ovary dry weights of *Pterostichus nigrita* treated in the same way as shown in figure 1.

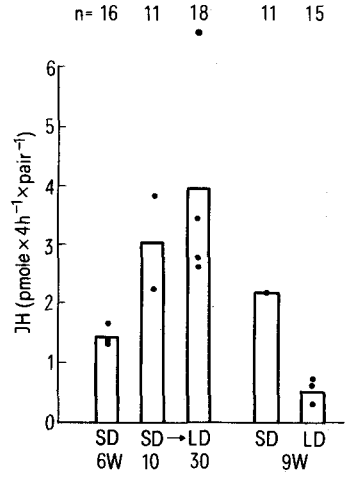

Figure 3. JH synthesis by isolated corpus allatum of male *Pterostichus nigrita*. Treatment of males was as described in figure 1.

ecdysteroids on maturation of female beetles could recently be ruled out[2].

In *P. nigrita,* photoperiods are only perceived by means of the compound eyes[7]. In an unknown way the day-length stimulates the neurosecretory cells (NSC) of the pars intercerebralis to produce and release paraldehydfuchsin (PAF) stainable material. This neurosecretion is transported along the nervi corporis cardiaci I (NCC) to the corpora cardiaca and may regulate the CA activity. In SD beetles this PAF positive material is present in large amounts in the brain; however, there is little transport along the NCC I. In females maturing after transfer from SD into LD, transport of neurosecretion could be demonstrated.

It can be concluded that SD photoperiods mainly stimulate activity of the NSC in the pars intercerebralis while LD allows transport of this accumulated material. Moderate LD (e.g. LD 16/8) stimulate both production and release of neurosecretion.

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Hormone content of mouse pancreatic islets subjected to different in vivo and in vitro functional demands[1]

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Summary. Mice treated for 4 days with tolbutamide displayed decreased serum glucose values with a concomitant decrease of their islet insulin content. Mouse islets cultured for 1 week at a low (3 mM) or a high (28 mM) glucose concentration contained less insulin than non-cultured islets and islets cultured at a medium (11 mM) glucose concentration. All groups of cultured islets contained more glucagon than non-cultured islets. The somatostatin content of high- and medium-glucose cultured islets was higher than that of freshly isolated islets.

During recent years much attention has been paid to pancreatic B-cells subjected to widely varying functional demands. Such experiments have been performed in vivo by starving experimental animals[3] or by treating them with, for example, sulfonylureas for a prolonged time period[4]. Alternatively, islet culture techniques have been used for assessing the in vitro effects of different supplementations of the culture media, i.e. glucose or amino acids[5,6]. The present study was undertaken to determine the hormone (insulin, glucagon and somatostatin) content of islets isolated from starved mice or mice treated for several days with tolbutamide. Furthermore, we determined the hormone content of islets maintained in culture for 1 week at different glucose concentrations.

Materials and methods. Collagenase (type CLS, 150–200 U/mg) was obtained from Worthington Biochem. Corp., Freehold, New Jersey, USA. Tissue culture medium (RPMI 1640 either glucose-free or containing 11 mmoles/l glucose) was supplied by Flow Laboratories Ltd, Irvine, Ayrshire, Scotland. Calf serum and Hanks' solution were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden. ^{125}I-insulin and ^{125}I-Tyr^{1}-somatostatin were from New England Nuclear, Dreieich, West Germany. Anti-insulin serum (code No. 65-101; lot No. GP20) was from Miles Laboratories, Inc., Elkhart, Ind., USA. ^{125}I-porcine glucagon, anti-porcine glucagon rabbit serum (K4023), crystalline mouse insulin and crystalline porcine glucagon were all obtained from Novo A/S, Bagsvaerd, Denmark. The anti-somatostatin serum used (R 141) was a gift from Dr R.P. Elde, University of Minnesota, Minneapolis, USA, and has been characterized elsewhere[7,8]. Crystalline ovine somatostatin was from Beckman, Geneva, Switzerland. Tolbutamide (Rastinon®) was purchased from Hoechst, Frankfurt, FRG. Other chemicals used were all of analytical grade.

Adult, male NMRI-mice (Anticimex, Sollentuna, Sweden) were used throughout the study. The animals were killed at about 08.00 h by decapitation and pancreatic islets isolated by collagenase digestion as described previously[9]. The islets were either transferred to culture dishes (see below) or directly homogenized by sonication for 30 sec in 500 µl acid ethanol (15 ml 12 moles/l HCl in 70% ethanol) and extracted over-night at +4 °C. Extracts were stored at −20 °C before the hormone assays.

For the in vivo experiments tolbutamide was injected i.p. as a 5% (w/v) solution (Rastinon®) to give a dose of either 125 or 300 mg/kg b.wt twice a day for 4 days. Control mice were injected with a 0.9% (w/v) sodium chloride solution. Another group of mice was starved with free access to drinking water for 60 h. Immediately before decapitation the orbital vein plexus was punctured and blood drawn for determination of the glucose and insulin concentrations.

For the in vitro experiments pancreatic islets were isolated from mice, which had been starved over-night prior to experimentation. The islets were transferred to Petri dishes for free-floating culture[3] at a glucose concentration of 3, 11 and 28 mmoles/l, respectively. The culture medium consisted of RPMI 1640 supplemented with 10% (v/v) calf serum and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). The dishes were incubated at +37 °C in a gas phase consisting of 5% CO_2 in humidified air and the medium was changed on the 3rd and 5th days of culture. After 7 days the islets were harvested, homogenized and extracted as described above.

Insulin, glucagon and somatostatin contents of the islet extracts as well as the serum insulin concentrations were all determined by means of radioimmunoassay procedures[7,8,10,11], separating free and antibody-bound hormone by ethanol precipitation. Serum glucose was determined using a Beckman Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, Calif., USA).

Results are given as means ± SEM with the number of experiments given in parentheses. For statistical analyses Student's t-test was used.