capillary tubes 20 h after ligation of the efferent ducts[2]. Levels of T and DHT were measured by radioimmunoassay after chromatographic separation on Sephadex LH-20 microcolumns[4]. At all ages examined, concentration of T in RTF (table) was within the range previously recorded in adult males of the same strain[2,4]. In contrast, DHT levels in RTF were substantially higher in immature than in adult rats (table).

The present results indicate that, in developing male rats, adult levels of T in RTF and thus presumably also in the seminiferous tubules are already attained at the age of 30 days, i.e. before any appreciable increase in peripheral T levels. This suggests that the correlation of spermatogenic activity with testicular but not peripheral T levels which was previously demonstrated in hypophysectomized, hormone-treated rats[2,4] may hold also during the course of normal sexual development.

Because plasma FSH levels in the male rat increase before sexual maturation[8] and FSH has been shown to stimulate the uptake of T by seminiferous tubules[9], we postulated that FSH may be responsible for the apparent preferential transport of T to the tubules and RTF in the immature male. However, when the effect of FSH (50μg of ovine FSH daily for 12 days) on the concentration of T in RTF was examined in adult hypophysectomized rats treated with 2 mg of T propionate or pregnenolone per day, there were no significant differences between FSH-injected and saline-injected control animals in the levels of T in RTF (T propionate group: 30.1 ± 14.8 vs 24.0 ± 9.4 ng/ml; Pregnenolone group: 17.0 ± 7.3 vs 13.2 ± 5.1 ng/ml). It is therefore unlikely that FSH is responsible for the observed distribution of T in immature animals. Instead, the presence of a facilitated diffusion carrier in the walls of the seminiferous tubules, as suggested by Setchell[10] may account for preferential accumulation of T in the tubules under conditions when total testicular T output is low, e.g. during early stages of sexual maturation or in hypophysectomized animals injected with precursors of androgenic steroids[4].

Increased concentration of DHT in RTF from immature rats is not unexpected in view of the well-documented increase in the activity of testicular 5α-reductase during this stage of development[6,11]. However, the possible physiological role of elevated DHT levels in the seminiferous compartment of the testis before sexual maturation remains to be determined.

Concentration of testosterone (T) and 5 α-dihydrotestosterone (DHT) in rat rete testis fluid during sexual development (means ± SE)

Age (days)	n	T (ng/ml)	DHT (ng/ml)
30	8	26.3 ± 11.3	42.5 ± 18.5
41	7	48.9 ± 7.4	15.6 ± 8.4
50	4	30.4 ± 11.8	7.8 ± 1.8
62	4	50.2 ± 11.1	3.7 ± 0.4
79	3	47.8 ± 9.0	2.9 ± 0.7
130	5	49.5 ± 12.3	2.4 ± 0.3

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Plasma testosterone and dihydrotestosterone in normal and abnormal pregnancy

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Summary. The concentration of plasma testosterone (T) and dihydrotestosterone (DHT) was determined in 2 groups of nonpregnant and pregnant women. The 1st group consisted of normal women and the 2nd of women with recurrent pregnancy disorders of unknown etiology. Significantly higher concentration of plasma DHT in nonpregnant women from the 2nd group was found (44.9 ± 22 ng/100 ml) as compared to nonpregnant normals (24.2 ± 5.2 ng/100 ml), $p < 0.01$. There was no difference in the concentration of plasma T between the groups studied ($p = 0.165$).

The hormonal status of women with recurrent pregnancy disorders with regard to estradiol and progesterone has been described previously[2-4], but the hormonal levels of other steroids such as testosterone (4-androsten-17β-ol-3-one) and dihydrotestosterone (5α-androstane-17β-ol-3-one) have not been tested. Since the androgens are involved in hormonal changes during the gestation period[5], it seemed worthwhile to determine the level of T and DHT in women with recurrent pregnancy disorders of unknown etiology.

Materials and methods. The control group consisted of women with no record of gestational disorders. They were classified in 4 subgroups as follows: a) nonpregnant normals; b) pregnant normals - 1st trimester; c) pregnant normals - 2nd trimester; d) pregnant normals - 3rd trimester. The experimental group consisted of women with recurrent gestational disorders of unknown etiology and they were grouped in the same way as normal women. Each woman had at least 3 pathologic pregnancies, predominantly spontaneous abortions[6]. Women with recurrent gestational disorders which could be related to abnormal glucose tolerance during pregnancy, to diabetes mellitus, to ABO and/or Rh sensitization, or to infections with *Toxoplasma, Listeria* or cytomegalovirus were not considered in this study. The average age of the normal controls was 25.2 years, and of women from the experimental group

Table 1. The mean plasma T- and DHT-concentration (ng/100 ml) in nonpregnant and pregnant women of control group and group with recurrent abnormal pregnancies (RAP)

Group	Testosterone range and mean ± SD	Dihydrotestosterone range and mean ± SD
Ia Nonpregnant normals	17–47	15–36
	28.7 ± 9.1	24.2 ± 5.2
	(N = 17)	(N = 15)
IIa Nonpregnant women with RAP	19–81	29–104
	33.7 ± 13.8	44.9 ± 22
	(N = 27)	(N = 11)
Ib Pregnant normals 1st trimester	30–264	14–68
	112 ± 71.1	46.6 ± 17.2
	(N = 9)	(N = 8)
IIb Pregnant women with RAP, 1st trimester	30–280	34–95
	102.4 ± 62.6	56.8 ± 18.3
	(N = 16)	(N = 11)
Ic Pregnant normals, 2nd trimester	43–255	28–160
	80.6 ± 49.3	55.1 ± 39.2
	(N = 16)	(N = 10)
IIc Pregnant women with RAP, 2nd trimester	22–265	18–232
	77.3 ± 49.4	55.5 ± 38.7
	(N = 41)	(N = 33)
Id Pregnant normals, 3rd trimester	33–169	40–200
	101.1 ± 46.6	82.6 ± 47
	(N = 11)	(N = 9)
IId Pregnant women with RAP, 3rd trimester	50–228	50–95
	132.9 ± 60.5	65 ± 18
	(N = 10)	(N = 5)

Table 2. The p-values of Student's t-test for all comparisons of T and DHT, mean concentrations in tested groups

	Ia	IIa	Ib	IIb	Ic	IIc	Id	IId
Ia	T	0.1646	0.0033	0.0002	0.0006	0.0000	0.0002	0.0001
	DHT	0.0076	0.0031	0.0001	0.0261	0.0002	0.0023	0.0005
IIa			0.0042	0.0003	0.0011	0.0000	0.0002	0.0001
			0.8534	0.2120	0.5063	0.2838	0.0490	0.0966
Ib				0.7488	0.2770	0.1908	0.7133	0.5320
				0.2567	0.5757	0.6455	0.0600	0.1236
IIb					0.2993	0.1641	0.9524	0.2480
					0.9013	0.8798	0.1582	0.5369
Ic						0.8176	0.3039	0.0363
						0.9761	0.2096	0.5496
IIc							0.1560	0.0127
							0.1370	0.5879
Id								0.2163
								0.6254

27.7 years. T and DHT were estimated in both groups by radioimmunoassay in the same sample of plasma. Approximately 1000 cpm of ^{3}H-T and 1000 cpm of ^{3}H-DHT were added to 1 ml of plasma for recovery, and the plasma was extracted with 10 ml of ethyl ether. The dry residue of the extract was dissolved in 2 ml of ether and transferred to an Al_2O_3 microcolumn[7]. The first 5 portions of 1.6 ml 0.45% absolute ethanol in hexane were discarded, afterwards DHT was eluted with 2 portions of 1.6 ml 0.45% absolute ethanol in hexane, and subsequently T was eluted with 2 portions of 1.6 ml 2% absolute ethanol in hexane. The radioimmunoassay was carried out using a testosterone RIA kit (Biolab, Limal, Belgium). Since the antiserum for T had 100% crossreactivity for DHT, the same antiserum was used for DHT estimations, purchasing DHT standard and DHT-[1,2,4,5,6,7-^{3}H(N)], (NEN Chemicals). The interassay coefficient of variation was 12.3% and sensitivity was 4 ng/100 ml for T, and 16.2% and 6 ng/100 ml for DHT, respectively. The mean T and DHT values were calculated for each subgroup of women separately and compared using Student's t-test.

Results and discussion. The mean T- and DHT-values for all subgroups are shown in table 1, and the p-values of all comparisons in table 2. The normal nonpregnant women had a lower mean T-value as compared to nonpregnant women with recurrent pathologic pregnancies, but the difference was not significant. However, the level of DHT was highly significantly different considering the same 2 groups of women (table 1).

Significant increase of total plasma T concentration was found in normal and abnormal pregnancy (tables 1 and 2). The plasma DHT was also increased in normal pregnancy, but only slightly in pregnant women with recurrent pathologic pregnancies.

Our findings on T- an DHT-levels in nonpregnant and pregnant normal women are consistent with those of Rivarola et al.[8], August et al.[9] and Saez et al.[10]. The concentration of DHT and estradiol in males is negligible in comparison to the testosterone concentration. In women the situation is somewhat different, as the concentration of DHT and estradiol is of the same order of magnitude as the concentration of testosterone. It was indicated that DHT may be an effective peripheral androgen at least in certain tissues[11]. In bioassay studies DHT has considerable androgenicity having a higher affinity for the nuclear protein than testosterone. The increased plasma DHT in women

with recurrent pregnancy disorders of unknown etiology indicates an altered production rate of DHT, but the biological significance of this phenomenon remains to be clarified.

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Acetylcholinesterase activity in an *Aedes aegypti* cell line

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Summary. Considerable acetylcholinesterase (AChE) activity was detected in an *Aedes aegypti* established cell line. The enzyme is blocked by 10^{-6} M eserine sulfate, displays excess substrate inhibition and slowly hydrolyzes butyrylthiocholine. A 2-fold stimulation of AChE activity was shown after 2 days exposure to 3×10^{-7} M β-ecdysone. AChE activity found in the fresh medium is the contribution of the fetal calf serum portion. A direct relationship between levels of serum and the AChE activity in the cultured cells was demonstrated.

The search for an understanding of the mode of action of the molting hormone at the cellular and biochemical level involves studies using various in vitro systems[2-6], including established insect cell lines[7-12]. Depending on the cell system, it has been demonstrated that the hormone causes either an increase in cell multiplication rate[7,9] or an extensive growth arrest[9,12]. In general, the hormonal effect was accompanied by morphological alterations[10]. Decreased RNA and DNA synthesis as well as changes in the electrophoretic profile of proteins were also detected[13,14]. Cherbas et al.[15] were the first to report that physiological levels of β-ecdysone induced acetylcholinesterase (AChE) activity in Kc cell-line of *Drosophila melanogaster*. Subsequently other accounts of the same phenomenon of induction in *Drosophila* lines were published[14,16]. It is of great significance that specific, well known and thoroughly investigated enzymes are induced by an insect hormone. The implications might be far reaching concerning major questions in insect endocrinology. The phenomenon of AChE induction offers an approach to elucidate the mode of action of β-ecdysone at the molecular level with ramifications to hormone receptors and the complex biochemical events leading to expression of AChE gene(s).

This report presents evidence for the existence of AChE in a mosquito cell line and deals with the effect of β-ecdysone on the enzyme activity. The presence of high AChE activity in the medium is discussed with regard to the recent finding of enzymatic induction by ecdysone in *Drosophila* cell lines.

Materials and methods. *Aedes aegypti* cultured cells[17] were grown in a medium supplemented with 10% fetal calf serum (GIBCO, Grand Island, N.Y.) in plastic flasks at 28 °C and subcultured weekly. For experimental purposes the cultured cells were maintained in disposable petri dishes (3.6 cm in diameter) and kept in a humidified 5% CO_2 atmosphere at 28 °C. To harvest the cells, the medium was removed and the culture was washed twice with a salt solution (0.1 M NaCl, 0.007 M KCl, 0.001 M Na HCO_3, 0.007 M KH_2PO_4, 0.0009 M $CaCl_2$). The washed cells were detached from the dish by vigorous pipetting and collected. The cell suspension was spun at $1000 \times g$ for 5 min and washed in the salt solution. After centrifugation the cell pellet was homogenized (Glass-teflon hand homogenizer) in 0.1 M pH-7.0 Na-phosphate buffer containing 0.5 M NaCl, 0.25 M EDTA and 0.5% (v/v) Triton X-100. The homogenate was kept on ice for 30 min and centrifuged at $10{,}000 \times g$ for 20 min. The supernatant served as a source of the enzyme. The assay procedure followed essentially that of Ellman et al.[18]. The reaction mixture in a final volume of 2.0 ml was composed of 1.95 ml 0.1 M, pH 8.0 Na-phosphate buffer, 0.5 mM either acetylthiocholine iodide or butyrylthiocholine iodide as substrates and 0.3 mM dithiobisnitrobenzoic acid (DTNB). After precalibration, the reaction was started by adding 50 µl of the enzyme extract. The absorbance at 412 nm was monitored continuously at 25 °C using Perkin-Elmer double beam spectrophotometer. Protein levels were determined by Lowry's method[19]. Enzyme specific activity is expressed as nmoles/min/mg protein.

β-Ecdysone (Rhoto pharmaceutical, Japan) was dissolved in absolute ethanol and 10 µl of the solution were added to cultures to give a final concentration of 3×10^{-7} M.

Results and discussion. High activity of AChE was detected in *Aedes aegypti* cultured cells (figure 1). It remains con-

Effect of β-ecdysone on AChE activity in *Aedes aegypti* cultured cells

Culture* (days)	β-Ecdysone treatment (h)	AChE activity (% of control)
2	24	134
2	48	194
3	48	199

* Culture age when β-ecdysone (final concentration of 3×10^{-7} M) was added. The cells were thoroughly washed, harvested, extracted and the AChE activity was assayed colorimetrically according to the procedure described by Ellman et al.[18].