Plasma testosterone concentration in control and testosterone-treated chick embryos

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Summary. After testosterone propionate treatment (1 or 2 mg per embryo), plasma testosterone in chick embryos rises to 500 times higher than in control animals, and then progressively diminishes during the following days of incubation. These drastic changes in hormonal status after the TP treatment entails consequences that may be considered pharmacological rather than physiological.

Testosterone has been previously shown to be synthesized in gonads of both sex chick embryos[4-6] and has also been found in plasma[7]. The physiological role of androgen hormones in embryonic sexual and non-sexual differentiation remains, however, a controversial matter in the following examples: sexual differentiation of gonads, regression of the mullerian ducts, and growth and differentiation of the bursa of Fabricius. In all instances, the high doses of hormone used for experimental treatment raise the question of the physiological significance of the results. Is an embryo treated with 1 or 2 mg testosterone propionate (TP) in near-to-physiological conditions, or are its organs subjected to pharmacological doses of hormone?

In a preliminary attempt to clarify this question, we have measured the blood testosterone concentrations of the chick embryo treated with testosterone propionate by 3 commonly used modes fo administration: vascular area, allantois, and intra-muscular injection.

Materials and methods. The chick embryos were treated with testosterone propionate (Sterandryl, Roussel-UCLAF) using 2 different procedures. At 4 days of incubation embryos received 2 mg TP in an oil drop placed on the vascular area surrounding the embryo. At 14, 16 and 17 days of incubation, 1 mg TP was injected in oil solution either i.m. in the leg, or in the allantoic sac. Untreated embryos were used as controls.

The blood was collected from extraembryonic veins or arteries with a 27-gauge needle mounted on a plastic disposable syringe. Sodium citrate was used as anti-coagulant. After centrifugation (10 min, 3000 rpm) the plasma was separated from the pellet and then stored at $-20\,^{\circ}C$ until utilization for testosterone determination. The blood of embryos treated with TP at 4 days was drawn at 9 days of incubation, while in embryos treated at 14, 16 or 17 days, blood was drawn 2, 3, 4 or 6 days after treatment (see table for details). Each embryo was usually punctured only once, but in 1 series blood was repeatedly collected at 16, 18 and 20 days in the same embryos. In these latter cases, the sample of blood collected never exceeded 0.3 ml, that is 10% of the total blood volume at this stage[8].

Testosterone concentration was measured by radioimmunoassay according to the procedure previously described[9,10]. In brief, this determination requires a preliminary extraction with diethyl ether (4 vol.) and a partial purification on Sephadex LH 20 mini-column (benzene ethanol; 95/5 by volume). The antiserum was raised in rabbits againt 3-α-carboxymethyl oxime-BSA and generously donated by J. Adeline (Fondation de la recherche en hormonologie). The specificity of the assay, as well as the precision, were satisfactory; the detection limit was estimated as 0.02 ng/ml.

Results. The mean testosterone concentrations in male and female chick embryos did not differ at any stage; therefore, the values were pooled for each experiment.

As shown in the table, the mean concentrations of testosterone in control embryos are similar at 9 and 18 or 20 days (approximately 50 pg/ml) but have increased about 2-fold at 15–16 days. Both increase ($p < 0.01$) and decrease ($0.01 < p < 0.05$) are statistically significant.

After treatment with 2 mg TP at 4 days, testosterone concentration soars up to 21,000 pg/ml at 9 days of incubation, that is 400 times more than in the controls. Treatment with 1 mg at 14, 16 or 17 days induced also a high mean increase of plasma testosterone concentrations. When measured 2 or 3 days after TP injection, the testosterone concentrations ranged form 40 to 500 times above those of controls, depending upon the experimental conditions. Repeated determination of testosterone in embryos

	Age in days of incubation 4	9	14	15–16	17	18	20
Untreated embryos	-	53.8 ± 20.9 (n = 19) ♂: 55; ♀: 53	-	117 ± 72.4 (n = 19) ♂: 119; ♀: 115	-	45.8 ± 26.7 (n = 6) ♂: 42; ♀: 48	57.5 ± 26.0 (n = 8) ♂:60; ♀: 67
Testosterone propionate (TP) treated embryos	+ 2 mg on vascular area	21,000 ± 18,100 (n = 16)	-	-	-	-	-
			+ 1 mg in allantois	8,600 ± 4,100 (n = 5)	-	3,360 ± 1,390 (n = 4)	973 ± 829 (n = 3)
			+ 1 mg i.m.	9,020 ± 6,200 (n = 8)	-	1,870 ± 287 (n = 5)	158 ± 43 (n = 4)
				+ 1 mg in allantois	-	8,100 ± 8,840 (n = 2)	-
				+ 1 mg i.m.	-	1,910 ± 1,020 (n = 2)	-
					+ 1 mg in allantois	-	29,000 ± 3,320 (n = 2)
					+ 1 mg i.m.	-	3,570 ± 1,530 (n = 3)

Testosterone concentration (pg/ml) in plasma of 9- to 20-day control and TP treated chick embryos. Mean values ± SD. n: number of determinations. i.m.: intramuscular injection. Doses injected, mode of administration and testosterone concentration are given in the column of age corresponding to the time the treatment was applied or the blood collected. Embryos treated at 14 days were repeatedly punctured at 16, 18 and 20 days.

which were treated at 14 days and then punctured successively at 16, 18 and 20 days, shows that the mean concentrations decrease rapidly after the injection. The decrease rate appears to be slower after injection in allantois than after i.m. injection; 6 days after injection, testosterone concentration is 3 times higher than in controls if TP is applied i.m., but is 17 times higher when applied through the allantoic sac.

From the data presented in the table, the large individual variation at each stage both from the control and treated groups should be noted. The coefficient of variation may be over 50% (control at 15–16 days), or even close to 100% (15–16 days treated embryos).

Discussion. When compared to the only results published to date on the plasma concentrations in chick embryo[7], our data show both some similarities and some discrepancies. Like Woods et al.[7] we find a peak of testosterone concentration in control embryos around 15 days of incubation, followed by a sharp decrease. Values range from 50 pg/ml for the basic level to 120 pg/ml. The male-female corresponding mean values published by Woods et al. are 60–160 pg/ml of plasma. Testosterone concentrations reported here for control embryos are lower than in the young cockerel (1.5–3 ng/ml)[11] but very close to the basic level in the laying hen (100–250 pg/ml)[12]. Unlike Woods et al.[7], we did not find any difference in the mean plasma testosterone concentrations between male and female embryos. Given the above similarities, this discrepancy remains unaccountable. However, recent studies[5,6] indicate the testosterone production in female gonads is not inferior to male gonads before 18 days of incubation. The variability observed in the present study reflects large individual differences, commonly reported from RIA of steroid hormones[9–14] and is in contrast to the very small SD values reported by Woods et al. which would indicate unusually narrow individual variability.

After TP treatment, the mean plasma testosterone concentrations increase enormously and reach unphysiological levels. Moreover, the large individual variability ovserved and the rapid changes in concentration during the days following treatment bring about an uncertain and changeable hormonal status in the treated embryos.

From the results reported herein, one may conclude that treatment of chick embryo with 1 or 2 mg TP cannot reproduce physiological conditions. This points to pharmacological rather than physiological consequences for such treatments and, therefore, enlightens controversial matters such as the paradoxical effect of androgens on gonadal differentiation, the inhibition of development of the bursa of Fabricius and the regression of the mullerian duct.

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Leydig cell function in streptozotocin-induced diabetic rats

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Summary. Streptozotocin diabetic rats were infertile as a result of decreased Leydig cell function of the testes. The major changes found were: decreased number of Leydig cells and their spontaneous secretion of testosterone. No change in the receptors to LH on the Leydig cells was observed. LH was found to be obligatory for the regulation of Leydig cell function and fertility.

Reproduction disturbances in diabetic patients are well-known and have been described in recent years[1,2]. In diabetic animals, impaired fertility is accompanied by pathological changes in the testes and male accessory glands[3]. In our previous studies[4,5] we have demonstrated changes in the male reproductive tract and in the hormonal system. Induction of diabetes caused significant decrease in the levels of serum LH and testosterone, implying defective endocrine function of the testes. It was concluded that the depression of testicular function seen in diabetic rats was secondary to a partial block of LHRH and LH secretions 4. The purpose of the present study is to evaluate the changes in isolated Leydig cell function of the diabetic rat with respect to hCG-binding receptors and testosterone secretion in vitro.

Materials and methods. The rats used in this study were male albinos weighing 250–300 g. They were fed pelleted animal food (Ambar, Hadera, Israel) and tap water ad libitum. Diabetes was induced by injection of a freshly prepared solution of 15 mg streptozotocin (Calbiochem, USA) in 0.1 ml citrate buffer (10 mM, pH 4.5) into the femoral vein. The diabetic state was verified by glucosuria and blood glucose.

The following experimental groups of rats were studied: 1. normoglycemic rats; 2. diabetic rats; 3. diabetic rats injected s.c. daily with insulin (2 IU protamine - zinc insulin, Nordisk Insulin Laboratorium); 4. diabetic rats injected s.c. twice weekly with 15 IU hCG (Chorigon, Ikapharm); 5. diabetic rats injected with insulin plus hCG; 6. diabetic rats injected with testosterone oenanthate twice weekly (0.5 mg in 0.1 ml oil solution, Schering, FRG); 7. diabetic rats injected with insulin plus testosterone. Treatment began 1 day after induction of diabetes.

After 2 months of treatment, the rats were decapitated and the blood was collected as serum for testosterone estimation. The animals were autopsied and the testes were