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.

1 Acknowledgment. We would like to thank Mrs N. Jeanguyot (UNCEIA, Laboratoire d'hormonologie, Maisons Alfort, France) for her excellent technical assistance in the RIA procedure.
2 Present address: Department of Anatomy, The University of North Carolina at Chapel Hill, 111 Swing Building 217H, Chapel Hill, North Carolina 27514, USA.
3 Present address: Endocrine Laboratory, University of Miami, School of Medicine, Miami, Florida, USA.
4 J.E. Woods and E.S. Podczaski, Gen. comp. Endocr. *24*, 413 (1974).
5 A. Guichard, L. Cedard, T.M. Mignot, D. Scheib and K. Haffen, Gen. comp. Endocr. *32*, 255 (1977).
6 C.T. Teng and C.S. Teng, Biochem. J. *162*, 123 (1977).
7 J.E. Woods, R.M. Simpson and P.L. Moore, Gen. comp. Endocr. *27*, 543 (1975).
8 C. Kind, Anat. Embryol. *147*, 127 (1975).
9 M. Thibier, Acta endocr. *81*, 623 (1976).
10 M. Thibier, Thèse Doctorat ès Sciences, Univ. Pierre and Marie Curie, Paris 1977.
11 J. Culbert, P.J. Sharp and J.W. Wells, J. Reprod. Fert. *51*, 153 (1977).
12 R.J. Etches and F.J. Cunningham, Acta endocr. *84*, 357 (1977).
13 E. Paulke and E. Haase, Gen. comp. Endocr. *34*, 381 (1978).
14 C. Monet, B. Martin and M. Thibier, Annls Biol. Anim. Biochim. Biophys. *16*, 795 (1976).

Leydig cell function in streptozotocin-induced diabetic rats

G. Paz and Z.T. Homonnai

Soferman Institute for the Study of Fertility, Maternity Hospital, P.O.B. 7079, Tel-Aviv (Israel), 4 January 1979

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

Table 1. Effect of diabetes and hormonal treatment on; body weight; glycemia; testicular weight; number of isolated Leydig cells and testosterone concentration in testes of the experimental groups. Statistical significances are given in the text

Treatment	Group size	Body weight at autopsy (g)	Post prandial serum glucose (mg/100 ml)	Testes weight (g/100 b.wt)	Leydig cells (millions/ animal)	Testosterone Testis (ng/g tissue)	Serum (ng/ml)
Normoglycemic	7	246± 9	120± 3	1.09±0.03	34±5.8	110±16	2.02± 0.25
Diabetic (D)	5	143±31	364±59*	0.66±0.15*	12±4.7*	38± 8*	0.28± 0.14*
D+insulin	9	210± 8	122±13**	1.10±0.06	15±2.1*	49± 4*	1.1 ± 0.2**
D+hCG	6	228± 8	306±19	1.16±0.05	56±6.3**	194±36**	4.7 ± 0.5**
D+insulin+hCG	9	256±11	181±23	1.08±0.03	38±2.2**	260±25**	8.5 ± 1.5**
D+testosterone	5	170±15	380±61	0.56±0.11*	23±4.8	184±47**	29.3 ±13**
D+insulin+testosterone	6	275±18	141±12	0.47±0.04*	27±3.9**	134±18**	-

*Significantly different from the normoglycemic rats; **significantly different from the diabetic rats.

Table 2. Isolated Leyding cells of diabetic rats; hCG binding capacity and testosterone secretion

Number of animals	Normal control 7	Diabetic 5	P(t)*
Testosterone concentration in serum (ng/ml)	2.02 ±0.25	0.28 ±0.14	0.01
Testosterone secretion rate in vitro (ng/10^6 cells/h)	8.3 ±1.7	2.5 ±0.9	0.01
Total ^{131}I-hCG binding capacity (cpm/10^8 Leydig cells)	19.700±2.100	16.300±3.700	NS
^{131}I-hCG bound after 'cold chaser' (cpm/10^8 Leydig cells)	2.100±520	1.300±120	NS

Values are means±SEM. *P(t), Significant of differences by the t-test.

isolated and weighed. Leydig cells were isolated by the method described by Mendelson[6]. The isolated cells were incubated in medium 199 to determine spontaneous secretion of testosterone. Studies on the binding of ^{131}I-hCG were carried out on isolated Leydig cells using the method described by Tsuruhara et al.[7], values are reported as means±SE. Significance of differences was calculated using the t-test.

Results and discussion. Diabetes caused a weight loss of 42±13% (p=0.005) (table 1). Insulin partially prevented this weight loss. Weight loss was diminished by treatment with hCG or prevented by hCG or testosterone plus insulin. Hyperglycemia during diabetes was pronounced. Insulin normalized this diabetic state. hCG and testosterone exacerbated the diabetes of the rats. Addition of insulin to these rats improved and stabilized the glycemia of the rats. Glucosuria was found in all the diabetic rats which were not treated with exogenous insulin. Diabetic animals suffer from a severe suppression of the functions of the testis and the accessory glands of the reproductive tract[8,9]; as a result, their fertility is drastically decreased[10,4]. In animals with streptozotocin diabetes, the concentration of testosterone in the serum was severely depressed ($p < 0.01$), (table 2). A large and highly significant reduction in the population of Leydig cells was found in the testes of streptozotocin-treated animals (table 1). The ability of these Leydig cells to secrete testosterone in vitro was measured (table 2). It was found in the diabetic animals, the secretion rate was only 30% ($p < 0.01$) that of Leydig cells taken from the testes of the normoglycemic rats. Studies on the binding capacity of Leydig cells for iodine-labeled hCG revealed that both the total binding capacity and the 'specific' high affinity binding capacity did not differ significantly between normal and diabetic rats (table 2). This implies that, in diabetic rats, the Leydig cells are not stimulated by endogenous gonadotropins as are Leydig cells of normal rats. The effect of diabetes and treatment with insulin, hCG, testosterone and combinations of these drugs on testicle weight, number of isolated Leydig cells and testicular and serum testosterone is given in table 1. Insulin, although preserving the testicle weight, showed no beneficial effect on Leydig cell function. The number of Leydig cells as well as their steroidogenetic activity was reduced ($p < 0.001$) as in the diabetic rats. hCG treatment restored testicular weight and endocrine function. The number of Leydig cells was increased by 37% ($p < 0.0001$) as was the testosterone in testicular tissue ($p < 0.001$) and in serum ($p < 0.005$). Treatment with both insulin plus hCG enhanced testosterone production and markedly increased serum testosterone above that of the diabetic hCG treated rats ($p < 0.001$). Testosterone did not change the weight of the atrophic testes of the diabetic rats. A significant increase in the population of the Leydig cells in the testes was noticed ($p < 0.05$). The injected testosterone was predictably found in high levels both in the testes ($p < 0.02$) and in serum ($p < 0.05$). Insulin did not change testosterone effects on testicular function.

The regulation of Leydig cell function by hormonal mechanism depend upon the integrative actions of gonadotropins (LH, FSH and prolactin) and steroids (estrogen and androgen). This regulation is mediated through the modulation of Leydig cell receptors for LH. While low concentrations of LH are neccessary for maintenance of Leydig cell function, elevation of plasma LH by treatment with LHRH or exogenous gonadotropin (hCG) has a marked negative effect on LH receptors[11]. Nevertheless, LH is obligatory in the regulation of the number and function of the Leydig cells. The dose of hCG used in this study had no negative effects on the endocrine function of the testis; on the contrary, it caused a marked increase in the number and secretory capacity of the Leydig cells.

1 D. Klebanow and J. Macleod, Fert. Steril. *11*, 255 (1960).
2 A. Rubin, Am. J. Obst. Gynec. *76*, 25 (1958).
3 K. Schöffling, K. Federlin, H. Ditschuneit and E.F. Pfeiffer, Diabetes *12*, 519 (1963).
4 G. Paz, T.Z. Homonnai, N. Drasnin, A. Sofer, R. Kaplan and P.F. Kraicer, Andrologia *10*, 127 (1978).
5 G. Paz, T.Z. Homonnai, A. Harell and P.F. Kraicer, Israel J. med. Sci. *14*, 1073 (1978).
6 C. Mendelson, M. Dufau and K. Catt, J. biol. Chem. *250*, 8818 (1975).
7 T. Tsuruhara, E.V. Van Hall, M.L. Dufau and K.J. Catt, Endocrinology *91*, 463 (1972).
8 L.E. Hunt and D.W. Bailey, Acta endocr. *38*, 432 (1961).
9 A. Oksanen, Horm. Res. *6*, 138 (1975).
10 V.G. Foglia, J.H. Rosner, M. Cattaneo de Peralta Romas and B.C. Lema, Horm. Metab. Res. *1*, 72 (1969).
11 M.L. Dufau, A.J. Hsuch, S. Cigorraga, A.J. Baukal and K.J. Catt, Int. J. Androl. (suppl. 2), 193 (1978).