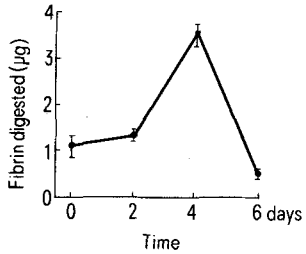
phosphatase (EC 3.1.3.2) and muramidase (EC 3.2.1.17) activities 1×10^7 cells were resuspended in 2 ml of 0.2% sodium deoxycholate in 1 mM tris buffer pH 7.4. The methods described by Nagata et al.[12] and by Prockop and Davidson[13] were used for the acid phosphatase and muramidase assays respectively.

Results and discussion. Following the addition of retinoic acid, HL 60 cells, which are predominantly promyelocytes, begin to differentiate into more mature myeloid cells as judged by their morphology and NBT reduction. Our data, not shown in the text, confirm those reported by Breitman et al.[8].

The effects of retinoic acid on the fibrinolytic activity of HL 60 cells are shown in the figure. The lytic activity was tested 2, 4, and 6 days following retinoic acid addition. Increase of the lytic activity was already present on the 2nd day; on the 4th day, when maximal morphological differentiation occurred, fibrinolytic activity also showed the highest level, while on the 6th day it had already dropped. Fibrinolytic activity was also tested, as shown in the table, following treatment for 4 days with DMSO, another inducer of myeloid differentiation in the HL 60 cell line[14]. No increase was found following the use of this compound. In the table the activities of acid phosphatase and muramidase of untreated cells and of cells treated for 4 days with retinoic acid and DMSO are also reported: both the inducers were found to produce an increase of acid phosphatase and a decrease of muramidase activity. Retinoic acid is known to increase the production of plasminogen activator of mouse teratocarcinoma cells[5]. Our data show that a similar effect is produced by this compound in HL 60 cells. In normal myeloid cells the fibrinolytic activity is known to increase in parallel to the maturation process, reaching a maximum at the metamyelocyte and granulocyte stages[15,16]. Thus the fibrinolytic activity can also be used as a parameter to evaluate myeloid differentiation. In our experiment we found that retinoic acid, but not DMSO, enhanced the fibrinolysis of human leukemic cells. On the contrary other parameters such as the morphology, the ability to reduce NBT and the activities of some lysosomal enzymes were affected in the same manner by retinoic acid and DMSO. Our findings suggest that retinoic acid provides a more reliable model for myeloid differentiation of the HL 60 cell line than DMSO.



Changes of fibrinolytic activity of HL 60 cells at different times following retinoic acid addition. µg of fibrin digested in 4 h at 37 °C/plate. (Mean values ± SD).

1 T. Moore, in: The Vitamins, vol. 1, 2nd edn, p. 245. Ed. W.H. Sebell and R.S. Harris. Academic Press, New York 1967.
2 W. Bollag, Eur. J. Cancer *8*, 689 (1972).
3 M.B. Sporn, N.M. Dunlop, D.L. Newton and J.M. Smith, Fedn Proc. *35*, 1332 (1975).
4 P.J. Becchi, H.J. Thompson, C.J. Grubbs, R.A. Squire, C.C. Brown, M.B. Sporn and R.C. Moon, Cancer Res. *38*, 4463 (1978).
5 S. Strickland and V. Mahdavi, Cell *15*, 393 (1978).
6 D.L. McCornick, F.J. Burns and R.E. Albert, Cancer Res. *40*, 1140 (1980).
7 K. Takenaga, M. Hozumi and Y. Sakagami, Cancer Res. *40*, 914 (1980).
8 T.R. Breitman, S.E. Selonick and S.J. Collins, Proc. natl Acad. Sci. USA *77*, 2936 (1980).
9 S.J. Collins, F.W. Ruscetti, R.E. Gallagher and R.C. Gallo, J. exp. Med. *149*, 969 (1979).
10 J.C. Unkeless, S. Gordon and E. Reich, J. exp. Med. *139*, 834 (1974).
11 M. Lassen, Acta physiol. scand. *27*, 371 (1952).
12 K. Nagata, E. Takahashi, M. Saito, M. Kuboyama and K. Ogasa, Exp. Cell Res. *100*, 322 (1976).
13 D.J. Prockop and W.D. Davidson, New Engl. J. Med. *270*, 269 (1964).
14 S.J. Collins, F.W. Ruscetti, R.E. Gallagher and R.C. Gallo, Proc. natl Acad. Sci. USA *75*, 2458 (1978).
15 J. Prokopowicz and H. Stormorken, Scand. J. Haemat. *5*, 129 (1968).
16 F. Ghezzo, A. Palumbo and L. Pegoraro, 5th int. conf. on synthetic fibrinolytic thrombolytic agents progress in fibrinolysis, Malmö 1980.

Androgen levels in the rete testis fluid during sexual development[1]

Mary E. Harris and A. Bartke

Astra Pharmaceutical Products, Worcester (Massachusetts 01613, USA), and Department of Obstetrics & Gynecology, The University of Texas Health Science Center, San Antonio (Texas 78284, USA), 5 August 1980

Summary. The concentrations of testosterone (T) and 5α-dihydrotestosterone were measured in fluid collected from the rete testis of immature and adult rats. The results indicate that adult levels of T are attained in the seminiferous tubules much earlier than in the peripheral circulation.

Production of spermatozoa in the adult male is thought to depend primarily on the locally high concentration of testosterone (T) in the testis. In the adult rat, concentration of T in the fluid collected from the rete testis (rete testis fluid, RTF) is approximately 20–30 times higher than in peripheral plasma[2]. Even though concentration of T in RTF is lower than in lumenal fluid collected from the seminiferous tubules[3], it correlates very closely with efficiency of spermatogenesis under a variety of experimental conditions[2,4] and thus appears to provide a valid estimate of T levels in the spermatogenic compartment of the testis. During sexual maturation of the male rat, plasma T levels increase sharply after the age of 30 days and reach adult values at 50–60 days of age[5]. However, spermatocytes already appear in the testes at 10 days of age and meiotic figures are abundant at 20 days of age[6]. It was therefore of interest to examine changes in androgen levels in the RTF of developing rats. To this end, concentrations of T and 5α-dihydrotestosterone (DHT) were measured in RTF from rats between 30 and 130 days of age. Younger animals were not included, because their testes secrete little if any fluid into the rete[7].

Male CD rats were purchased from Charles River Breeding Laboratories and samples of RTF were collected with glass

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

1 This work was performed at the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, USA and supported by the National Institutes of Health through grants HD12641 and HD12642. We thank Dr B.V. Caldwell for antiserum to testosterone.

2 M.E. Harris and A. Bartke, Endocrinology *95*, 701 (1974).
3 F.H. Comhaire and A. Vermeulen, J. Endocr. *70*, 229 (1976).
4 M.E. Harris and A. Bartke, Endocrinology *96*, 1396 (1975).
5 D.W. Knorr, T. Vanha-Perttula and M.B. Lipsett, Endocrinology *86*, 1298 (1970).
6 M.A. Rivarola, E.J. Podesta and H.E. Chemes, Endocrinology *91*, 537 (1972).
7 B.P. Setchell, T.W. Scott, J.K. Voglmayr and G.M.H. Waites, Biol. Reprod. suppl. *1*, 40 (1969).
8 R.S. Swerdloff, P.C. Walsh, H.S. Jacobs and W.D. Odell, Endocrinology *88*, 120 (1971).
9 A. Seilicovich and J.M. Rosner, Steroids Lipids Res. *3*, 214 (1972).
10 B.P. Setchell, J. Androl. *1*, 3 (1980).
11 E. Steinberger and M. Ficher, Endocrinology *89*, 679 (1971).

Plasma testosterone and dihydrotestosterone in normal and abnormal pregnancy

Melita Tajić, M. Gerenčer and A. Kaštelan[1]

Department of Medicine, Institut of Endocrinology and Tissue Typing Centre University Hospital Rebro, KBC, and Department of Animal Physiology, University of Zagreb, Kišpatićeva 12, 41000 Zagreb (Yugoslavia), 2 June 1980

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