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Testicular Blood Flow Rates in Prepubertal and Adult Rabbits Measured by ^{85}Krypton (38301)

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(Introduced by W. Hansel)

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The testis is highly vascularized, and interference with blood flow rapidly impairs testicular function (1). Numerous factors have been shown to influence testicular blood flow rates (2, 3). Lindner (4) reported increased blood flow rate per testis as bulls grew. Whether or not the increase was due to testicular growth, or whether the cellular proliferation characterizing onset of spermatogenesis at puberty placed an increased burden on the circulatory system was not specified. It was the purpose of the present study to determine if testicular blood flow rates varied between prepuberal and postpuberal male rabbits and whether blood flow in prepuberal bucks might be altered by gonadotropin treatment.

Material and Methods. Twenty Dutch male rabbits were assigned equally to one of the following treatment groups: (A) 12 week old controls, (B) 12 week old males injected subcutaneously (sc) twice daily for 17 days with 1.0 mg of luteinizing hormone (Armour–Baldwin PLH), (C) 12 week old males injected sc twice daily for 17 days with 0.3 mg of follicle stimulating hormone (Armour–Baldwin FSH-P), (D) 24 week old, and (E) 1–3 years old. Blood flow rates were estimated on Days 4 and 17 of the treatment period. Bucks were lightly anesthetized with sodium pentobarbital and anesthesia was maintained with ether. Testicular weights were estimated by the method of Paufler *et al.* (5). Blood flow rates were estimated by the ^{85}Kr clearance technique (2). Intra-testicular injections were made with a 0.5 ml syringe attached to a 27 gauge needle. Approximately 0.05–0.10 ml of saline solution containing 100–200 μCi ^{85}Kr was injected midway between the poles of the testis. Care was taken to avoid the area of the mediastinum. All injections were made with the buck in a ventilation hood to remove the expired ^{85}Kr. The side of the first injection was random. A scintillation probe (Nuclear–Chicago) with a 2.5 cm sodium iodide crystal was used to monitor the "wash-out" of the radionuclide until background levels were reached or for a maximum of 20 min. The output from the probe was led into a linear ratemeter and continuously recorded on a potentiometric recorder. Total activity was corrected for background activity and plotted as counts per minute (CPM) against the time postinjection on semilogarithmic graph paper. A straight line was drawn through these points and extrapolated to the time of injection. The biological half-line was determined and testicular blood flow rate (ml/g tissue/min) estimated as previously described for ram testes (2).

Immediately after the blood flow estimations a biopsy was taken from the left testis on Day 4 and from the right testis on Day 17 by the procedure of McFee and Kennelly (6). Chalkley's (7) method was followed to estimate the proportion of total testicular volume falling into the following four categories: tissue within a seminiferous tubule, space within a tubule, interstitial tissue and nontubular space.

Results and Discussion. In a preliminary experiment, compressing the spermatic cord blocked the clearance of ^{85}Kr from the testis, providing additional evidence that the clearance of ^{85}Kr was a function of blood flow. Also, it was noted that the site of injection influenced the estimated blood flow rates, being significantly higher ($P < 0.10$) when injections were made in the caput end compared to the caudal end of the testis. There was no difference ($P > 0.10$) in

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TABLE I. Effect of Age and Treatment on Rabbit Testicular Blood Flow Rates.

Treatment group	Age	Treatment[a] (daily for 17 days)	Blood flow[b,c] (ml/g tissue/min) Day 4	Day 17	Mean
A	12 wk	None	0.123	0.125	0.124
B	12 wk	2.0 mg LH	0.118	0.082	0.100
C	12 wk	0.6 mg FSH	0.115	0.144	0.130
D	24 wk	None	0.123	0.139	0.131
E	1–3 yr	None	0.114	0.116	0.115
		Mean:	0.119	0.121	0.120[d]

[a] Half of the daily dose given subcutaneously at 12 hr intervals.

[b] Group means derived from the average of two (left and right sides) testicular estimates per buck.

[c] There were no differences ($p > .10$) due to treatment group or period. The treatment × period interaction was significant ($p < .10$).

[d] Overall mean of 80 measurements (20 rabbits × 2 testes × 2 periods).

estimated blood flow rates between the left and right testes. Subsequently, all injections were made in the central portion of the testis.

Mean blood flow rate of 80 measurements (20 rabbits × 2 testes × 2 periods) was 0.120 ml/g tissue/min (Table I). This value is between the 0.228 and 0.084 ml/g min reported previously for the dog and conscious ram (2), respectively. Total blood flow to the testis increased greatly between 12 and 24 weeks, as the testis increased in size. There was no difference ($P > 0.10$) in blood flow rate/g tissue due to age. The correlations between blood flow and testis size for left and right testes were 0.92 and 0.90. Thus, rate of blood flow to the testis appears to be mainly a function of testis size. Blood flow rate/g did not differ among gonadotropin treatments, period or testis. The treatment × period interaction was significant ($P < 0.10$), with blood flow/g of tissue reduced in Group B and increased in Group C on Day 17 compared to Day 4 of treatment. This result is suggestive of an FSH stimulation, since not only did flow rate/g go up, but testis size also increased proportionately more in Group C (Table II).

Testicular characteristics are shown in Table II. Testis size was considerably larger in the older animals and there was a tendency for relatively less interstitial tissue to be present. However, blood flow rate was apparently primarily related to testicular size. Total blood flow was correlated with the presence of sperm on the two sides (0.77 and 0.79), as a result of its relationship to testis size in the different age groups.

Gonadotropin treatment for 17 days at the levels used in this experiment failed to stimulate

TABLE II. Effect of Age and Treatment on Rabbit Testicular Characteristics.

Treatment group	Sperm present	Testicular wt[a] (g) Day 4	Day 17	Mean	Seminiferous tubules[b] (%) Day 4	Day 17	Mean	Interstitial tissue[b] (%) Day 4	Day 17	Mean
A	No[c]	0.55	0.90	0.73	80.4	79.4	79.9	17.8	15.5	16.7
B	No	0.68	0.96	0.82	77.1	79.6	78.4	18.0	18.6	18.3
C	No	0.52	0.88	0.70	74.8	79.6	77.2	22.2	17.8	20.0
D	Yes[d]	2.03	2.18	2.11	81.2	80.5	80.9	15.2	13.7	14.5
E	Yes	3.37	4.03	3.70	85.7	81.9	83.8	11.5	15.5	13.5

[a] Group mean derived from combined estimated weights of left and right testes.

[b] Percentage of total testicular volume.

[c] Sperm present in seminiferous tubule of one buck.

[d] Sperm not present in seminiferous tubule of one buck.

spermatogenesis as found previously in the immature boar (8). Linder (4) found no effect of gonadotropin treatment on blood flow in bulls, although testosterone levels were elevated following administration of human chorionic gonadotropin.

Sperm first appear in the ejaculates of male rabbits at about 17 weeks of age (9), while ova can be produced by gonadotropin administration in females at 12 weeks of age (10). Gonadotropin levels used in the present study had no effect on any of the criteria examined although these levels will stimulate superovulation in the female (11).

Summary. LH and FSH given to 12-week old rabbits for 17 days failed to alter spermatogenesis or blood flow. As testes grew in gonadotropin treated males, or controls ranging in age from 12 weeks to 3 years, the increase in total blood flow corresponded to the increase in testis size. Overall, the mean testicular blood flow rate for 80 measurements was 0.120 ml/g tissue/min.

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