EFFECT OF ELEVATED AMBIENT TEMPERATURE ON TESTIS AND BLOOD LEVELS AND IN VITRO BIOSYNTHESIS OF TESTOSTERONE IN THE RAM

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SEASONAL differences in spermatogenesis, with minimal activity occurring during the summer months, have been consistently observed in rams. This condition is usually referred to as "summer sterility", and results primarily from an increase in environmental temperature (Dutt, 1960; Lodge and Salisbury, 1970; VanDemark and Free, 1970).

Although little doubt remains that mammalian spermatogenesis is a heat labile process, it is not clearly established whether or not the steroidogenic function of the testis is affected by elevated testicular temperature. Histological examination of cryptorchid testes suggests an increase in Leydig cells (Clegg, 1961), but indirect measures of androgen secretion have given contradictory results (VanDemark and Free, 1970). Studies involving measurement of androgen levels in cryptorchid testes of rams and bulls (Skinner and Rowson, 1968), testosterone secretion in spermatic venous blood of cryptorchid dogs (Eik-Nes, 1968) and testosterone biosynthesis by cryptorchid rat testes in vitro (Inano and Tamaoki, 1968) all indicate decreased steroidogenic function of mammalian testes which have been restricted to the body cavity.

Cryptorchidism generally results in only partially reversible, or irreversible, damage to the spermatogenic epithelium, whereas summer sterility is a transitory condition, suggesting that the endocrine response to the two conditions may not be the same. This study was undertaken to determine testis tissue and blood testosterone levels and in vitro biosynthesis of testosterone as affected by elevated ambient temperature.

Materials and Methods

Experimental Animals. A total of 34 yearling rams of mixed breeding was used in the various portions of this study. The rams were randomly divided into two groups and subjected to outdoor temperatures (control) or to 2 weeks of elevated temperature.

Control rams were maintained in a conventional open shed under Ohio spring conditions. Treated animals were maintained in the room described by Johnson, Gomes and VanDemark (1969) and subjected to peak temperatures of 32 C. Night time cooling to 28 C was allowed to occur and light was maintained to conform to existing outdoor daylight hours (Johnson et al., 1969). After a 14-day period of exposure, treated animals were immediately killed for collection of testes or subjected to surgery for collection of spermatic venous blood.

Sample Collection. Animals used for collection of spermatic venous blood were given local anesthesia (procaine hydrochloride) in the area of the inguinal canal and the left spermatic vein was cannulated as described by Free and VanDemark (1968). Animals were allowed at least 1 hr. postoperative recovery time before blood was slowly collected through the cannula into a heparinized syringe. Blood samples were centrifuged in a refrigerated centrifuge and the plasma frozen until thawed for assay.

When testis tissue was to be used, animals were sacrificed and the testes were immediately removed and chilled. The portion of each testis to be used for testosterone assay was minced with a meat grinder in a cold (3 C) room and, after duplicate samples were taken for dry weight, frozen and stored at -20 C until thawed for assay. Tissue to be used for in vitro incubations was sliced with a Stadie-Riggs hand microtome.

Testosterone Assay. Samples for testosterone assay were thawed and, after addition of a

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tracer amount of testosterone-4,14C (0.01 μCi; 0.05 μg), the sample was extracted with diethyl ether (plasma) or ethyl acetate (tissue). Tissue extracts were dried and resuspended in diethyl ether. From this point, all samples were treated alike.

Either fractions were successively washed with distilled water and 1N KOH, evaporated to dryness, and taken up in a small volume of chloroform:methanol (2:1 v/v). These samples were then applied to glass thin-layer chromatography plates coated with silica gel G and chromatographed in two successive one-dimensional solvent systems: benzene-ethanol (9:1) followed by ethyl acetate-cyclohexane (1:1). Areas of the gel corresponding to testosterone standards were eluted with chloroform:methanol (2:1 v/v; 3 x 2 ml) and the combined eluates were taken to dryness under nitrogen.

The purified sample extract was dissolved in 10 μl of chloroform:methanol (1:1 v/v), and 1 μl aliquots were assayed by gas-liquid chromatography using a Gas-Chrom Q column coated with 3% QF-1 and a flame ionization detector. Testosterone was quantified by triangulation of the free testosterone peak. Verification of identity was accomplished by comparing RF and retention times of the unknown compound and its derivatives to standard testosterone, testosterone acetate and the trimethylsilyl ether of testosterone. Aliquots of each concentrated sample were assayed for radioactivity in a liquid scintillation counter and correction was made for procedural losses for individual samples.

In Vitro Incubation. Testis tissue slices weighing 300 to 500 mg were placed in four flasks per testis. Each flask contained 5 ml of Krebs-Ringer phosphate buffer (pH 7.2), 10 mg of glucose and either cholesterol-4,14C (2 μCi/flask) or pregnenolone-7α,3H (10 μCi/flask). The samples were incubated for 2 hr. at 35 °C under an atmosphere of 95% O2 and 5% CO2. After incubation, the flasks were quick-frozen to stop metabolic activity and stored at —20 °C until assay.

The contents of each incubation flask were homogenized with a motor driven pestle and, after addition of 100 μg of unlabeled testosterone, were extracted with ether. Extraction, chromatography, derivative (acetate) formation and assay of radioactivity in testosterone were carried out as described by Connell, Connell and Eik-Nes (1966). Radioactivity was expressed as dpm radioactivity per gram of dry wt. of incubated tissue.

In some cases, the numbers of samples from control and treated animals differed, due to loss of samples or unsuccessful cannulation of veins. All sample means were compared for significance using the t-test.

Results and Discussion

The effects of elevated ambient temperature on testis weight, testosterone levels in tissue and blood, and ability of testicular tissue to form testosterone from labeled precursors are summarized in table 1. In all but one comparison, measured values from heat-treated rams were significantly lower than those in control tissues.

The decrease found here in testis weight of rams subjected to higher temperatures has also been reported by others, and was not unexpected in view of the heat induced loss in histologically apparent cellular materials (De-

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Treated</th>
</tr>
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<tbody>
<tr>
<td>Testis weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet wt. (g)</td>
<td>9</td>
<td>120±5</td>
</tr>
<tr>
<td>Dry wt. (g)</td>
<td>9</td>
<td>12.4±0.7</td>
</tr>
<tr>
<td>Testosterone levels</td>
<td></td>
<td></td>
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<tr>
<td>μg/g testis dry wt.</td>
<td>7</td>
<td>1.09±0.52</td>
</tr>
<tr>
<td>μg/testis</td>
<td>7</td>
<td>14.6±3.6</td>
</tr>
<tr>
<td>μg/100 ml spermatic venous plasma</td>
<td>7</td>
<td>8.2±4.4</td>
</tr>
<tr>
<td>In vitro incorporation of label into testosterone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^6 dpm from cholesterol-4,14C/g testis dry wt.</td>
<td>6</td>
<td>1.50±0.95</td>
</tr>
<tr>
<td>10^5 dpm from cholesterol-4,14C/testis</td>
<td>6</td>
<td>1.73±1.11</td>
</tr>
<tr>
<td>10^5 dpm from pregnenolone-7α,3H/g testis dry wt.</td>
<td>6</td>
<td>3.57±0.67</td>
</tr>
<tr>
<td>10^5 dpm from pregnenolone-7α,3H/testis</td>
<td>6</td>
<td>4.04±0.60</td>
</tr>
</tbody>
</table>

* No. of animals.
** Significantly different than controls (P<.05).
* * Significantly different than controls (P<.01).
Subcellular or biochemical alterations during heat treatment, however, are not so readily apparent. Histological examination suggests that only spermatogenic elements disappear, and that interstitial materials remain unchanged or increase in numbers and volume (Clegg, 1961), but the data reported in table 1 suggest that the interstitial elements do not function as undamaged tissues.

The control value for testosterone concentration in testicular tissue (table 1) is similar to the levels reported for the rat (Hashimoto and Suzuki, 1966; Resko, Feder and Goy, 1968) and rabbit (Ewing and Elk-Nes, 1966). The concentration was decreased to one-third of control levels after 2 weeks of exposure to high ambient temperature. If elevated temperatures had no effect on interstitial elements or their hormone production, one would expect an increase in testosterone concentration, proportional to the compacting effect on Leydig cells of loss of germinal elements. To the contrary, the heat treatment decreased both concentration and content of testosterone in the testis.

The concentration of testosterone in spermatic venous blood plasma from control rams was 8.2±4.4 µg/100 ml (table 1). In his studies, Lindner (1961, 1963) found a concentration of testosterone ranging from 1.7 to 10.5 µg/100 ml in spermatic venous blood of seven rams, and observed that levels were extremely low during the nonbreeding season. In the present study, spermatic venous testosterone concentrations were decreased markedly by the imposition of a treatment simulating summer temperature conditions; treated animals exhibited relatively less between animal variation than did the controls.

Although concentrations of testosterone in spermatic venous blood are a function of blood flow rate as well as hormone production by the testis, it is unlikely that the values in table 1 can be explained by increases in flow rate, since summer aspermia in rams (Lindner, 1961), cryptorchidism in dogs (Elk-Nes, 1968) and heat treatment of ram testis in vivo or in perfusion all resulted in decreased or unchanged rates of blood flow through the testis (Setchell, 1970).

As shown in table 1, the incorporation of pregnenolone-3H into testosterone in vitro was significantly lower in tissue from treated rams. Although the incorporation of cholesterol-14C into testosterone was not significantly decreased when expressed on a weight basis (table 1), correction for testis weight differences revealed that treated testes could convert significantly less cholesterol to testosterone than controls, under the in vitro conditions employed.

If the decreased testosterone concentrations in testes and spermatic venous blood found in this study were reflections of indirect physiological conditions existing as a result of high temperature, and not of reduced gonadotrophin levels or decreased capability of the Leydig cell to function, one might expect the effects to be overcome by in vitro incubation of the tissues. If this were true and if interstitial tissues were not affected by heat treatment, the Leydig cells should produce testosterone in vitro proportionally to their concentrations; i.e., tissue from heat-treated rams should contain more Leydig cells per unit of weight (since germinal elements have decreased) and should therefore produce more testosterone per unit of weight. In view of indirect evidence indicating increased levels of gonadotrophin during heat treatment of testes (Clegg, 1961), and the data reported here suggesting decreased biosynthesis, direct effects of heat treatment on the interstitial tissues cannot be disregarded.

Thus, all of the endocrine parameters measured in this study—testicular tissue testosterone, spermatic venous testosterone, and in vitro biosynthesis of testosterone—indicate that elevated ambient temperature is detrimental to Leydig cell function in the ram.

**Summary**

Thirty-four yearling rams were subjected to Ohio spring conditions (control) or elevated temperature for 2 weeks in a temperature controlled room (treated).

Rams had significantly smaller testes after heat treatment, compared to controls. Concentration of testosterone in testis tissue was 1.09±0.52 µg/g dry wt. for controls, but only 0.35±0.15 µg/g for testes from treated rams (P<.05). Similarly, spermatic venous blood testosterone decreased (P<.05) from 8.2±4.4 to 1.9±0.6 µg/100 ml plasma following treatment. Testis tissue from treated rams also incorporated less labeled precursor (cholesterol and pregnenolone) into testosterone in vitro than tissue from control rams.

**Literature Cited**


