THE STEROID PRODUCTION OF THE
TESTICLES AND ITS RELATION TO NUMBER AND
MORPHOLOGY OF LEYDIG CELLS

By

K. G. Tillinger, G. Birke, C. Franksson and L.-O. Plantin

The androgen-producing capacity of the testicles is widely considered to be confinned to the interstitial cells (Leydig cells). This view is based to some extent on animal experiments, but chiefly on the following observations:

1. The reputed existence of developmental and morphological similarities between the Leydig cells and those cells of the adrenal cortex that are assumed to produce androgenic hormones.

2. In patients with totally destroyed seminiferous tubules but with more or less normal numbers of Leydig cells, the secondary male characteristics, once developed, do not redevelop in the same way as they do in castrated subjects.

3. Patients showing seminiferous tubules with active spermatogenesis, but devoid of Leydig cells, present signs of reduced androgen production.

4. Testicular tumours of cells resembling the Leydig cells (interstitial cell tumours) are associated with an increased excretion of androgens in the urine. In boys they cause precocious pseudopuberty.

To those who examine histological specimens from the testicles it is soon evident that the testicular function with regard to androgen production, as reflected in hormone analyses and clinical observations, cannot be evaluated solely on the basis of Leydig cell counts. For instance, patients with signs of hypogonadism sometimes present quite an abundance of Leydig cells, which extend between the seminiferous tubules in massive confluent bands and sometimes, indeed, form adenoma-like masses.

If the Leydig cells are the site of testosterone production, then these patients with Leydig cell hyperplasia should not show any signs of reduced androgen
production, provided the steroid synthetisizing power of their Leydig cells is not impaired.

Could a better relation be obtained between the clinical and the hormone analytical findings on the one hand, and the histological findings, if not only the number but also the morphology of the Leydig cells were taken into account? No unequivocal answer to this question has yet been forthcoming, partly because it has not previously been possible to determine accurately the testicular role in the androgen production. Some attempts have been made to investigate this correlation (Teem, 1937; Goldzieher & Hamblen, 1947; Hooker, 1948; Howard et al., 1950; Hyman, 1950; Burt et al., 1954), but none of these studies have included determinations of the relative testicular production of 17-ketosteroids.

In such an investigation the use of cytochemical methods seems appropriate. However, not only are these complicated and hence unsuitable for routine use, apart from requiring special fixing methods; but also, there are still no reliable methods of demonstrating steroids histochemically (Albert & Leblond, 1946; Pearse, 1953), even though some investigators claim that their methods are specific (Bennet, 1940; Seligman & Ashbel, 1951).

An attempt therefore has been made to study the Leydig cells in detail as they appear in histological specimens, to classify them, and to find which types are most common in patients with good androgen production (Sniffen, 1950: investigation based partly on a paper of the same year together with Howard, Simmons & Albright).

On the basis of Sniffen’s description of the Leydig cells in normal testes, a rough and schematic classification has been made, with the following types:

A. Small fusiform or polygonal Leydig cells with finely granulated eosinophilic cytoplasm. Immature cells.
B. Medium-sized polygonal cells, mostly with finely granules but sometimes with a few coarser granules admixed (Fig. 1).
C. Large polygonal cells with finely granulated cytoplasm centrally round the nucleus, the peripheral zone being light and containing small droplets or vacuoles (endoplasmic and endoplasmic portions). Scattered coarser granules and a few pigment granules may be found.
D. Medium-sized polygonal cells with an abundance of somewhat coarse granules, often in clumps. Pigment is present, frequently in abundance. Vacuoles are common, and occasionally occur in such numbers as to give the cytoplasm a foamy appearance. Degenerative form (Fig. 2).
E. Small fusiform cells, mostly filled with pigment. Involutional form.

According to Sniffen, cell types B and C are the most active with regard to testosterone production, the others having a very slight steroid production or none at all.

Reinke’s crystalloids (crystalloid formations in the cytoplasm, first described by Reinke in 1896) are found in types B, C and D, though in greatly varying
Fig. 1.

Case 3. Showing a group type B Leydig cells with homogeneously granulated cytoplasm. In some places are groups of coarser granules and signs of clearing up in the peripheral zone. These cells are intermediate between types B and C. (Ladewig’s collagen tissue stain; \( \times 400 \)).

amounts: sometimes singly in an occasional cell, sometimes abundantly, and often with several in the same cell. Their role with regard to steroid production is still obscure.

The microscopic examination of the Leydig cell count gives, of course, merely a relative and rough estimate – primarily a measure of the area occupied by these cells in relation to the rest of the testicular tissue. In evaluating the function of the Leydig cells, therefore, the investigator must take into account the size of the testicles if he is to gain an idea of the total Leydig cell count. This count may be called the «absolute» number of Leydig cells.

Several methods have been reported for quantitative determination of the Leydig cells (Stieve, 1919; Romeis, 1921; Wagner, 1922; Benoit, 1922; Lundgren, 1926; Lundh, 1927; Saller, 1928; Sargent & McDonald, 1948), but most investigators of recent years consider that such methods do not yield much more information than can be gathered by an experienced person from a general study of specimens (Sand & Okkels, 1936; Hemphill et al., 1944; Sniffen, 1950).

With a view to correlating the number and morphology of the Leydig cells with the androgen production of the testicles, we investigated a series in which both steroid analyses and histological examinations were made. Having regard
Case 7. Leydig cells of type D with abundance of coarse pigment granules. Round the group of cells is a broad band of collagen tissue. In the upper part of the Leydig cell to the lower left is a shadow formed by a Reinke crystalloid. (Ladewig’s collagen tissue stain; × 400).

to the above mentioned considerations, the histological evaluation was concerned both with the »absolute« number and the appearance of the Leydig cells, in order to establish, if possible, which of these two factors is most consistent with the steroid production.

The testosterone-producing capacity of the testicles was evaluated on the following principles. After administration of testosterone, about 50 per cent of it is transformed and excreted as the 17-ketosteroids, androsterone and etiocholanolone (Callow et al., 1938; Dorfman et al., 1940; Dobriner et al., 1950; Gallagher et al., 1951; and Birke et al., 1954). No extensive transformation to other steroids is considered to occur. The decrease of androsterone and etiocholanolone found in the urine after castration provides, therefore, a measure of the steroid production of the testicles in the particular case.

M E T H O D S

Steroid Analysis. – The urine was hydrolysed with H₂SO₄ at pH 0.4 by boiling for 25 minutes, after which continuous extraction with ether proceeded for 16 hours. Following separation ad modum Girard, colorimetric determination of the 17-ketosteroids
was done by Gallo's modification (1940) of Zimmermann's method (1935). Correction for non-specific chromogens was made ad modum Gibson & Evans (1951). For chromatography of the 17-ketosteroids, Zygmunadowicz's method (1951) was employed, supplemented by a modification permitting identification of the steroids by infrared spectrography. This modified method has been described in detail in a previous paper (Plantin & Birke, 1954).

The urinary excretion of total 17-ketosteroids and of androstosterone and etiocholanolone was investigated three days before castration, and a mean value calculated. Any eventual reduction of these steroids was determined for three days postoperatively in order to obtain a reliable post-castration mean value; for such a reduction is considered to afford a criterion of the steroid production of the testicles.

Histological Examination. — Immediately after castration, the testicles were immersed in Stieve's fluid (76 parts saturated sublimate solution, 20 parts 40 per cent formalin, and 4 parts glacial acetic acid), though prior to this a long incision was made through the tunica albuginea to facilitate penetration of the fixative. After fixation for 2–7 days the testis was weighed and a transverse slice removed, then dehydrated in 95 per cent alcohol, absolute alcohol, benzene; following which embedding was done in a paraffin oven for 48 hours. Sections of 5 μ were stained with hematoxylin-eosin and by Ladewig's collagen tissue method, the latter of which also gives satisfactory nuclear staining.

MATERIAL

The investigation comprised 19 patients with prostatic cancer who had undergone therapeutic castration. Their ages ranged from 59 to 83 years. Four of them received stilbestrol for a relatively long period before operation.

The series is not a fully adequate basis on which to answer the question raised in the introduction, for all the patients were men of advanced age, and these usually show a reduced androgen production. It was not possible, however, to collect a series of younger patients for this kind of investigation.

RESULTS

The histology of the testicles was evaluated without knowledge of the results of the hormone analyses. An appraisal of this kind, like most attempts at functional determinations on the basis of microscopic pictures, is bound to be very crude. The results obtained here are shown in Table 1.

The hormone determinations are shown in Table 2. The 24-hour excretion of 17-ketosteroids is included to facilitate a comparison with other series.

In Table 1 a comparison is given of some characteristics of the testicles investigated and the reduced excretion of androgens resulting from castration. Although the testicular weight shows no distinct relation to the androgen excretion, the lowest weights are nevertheless found among cases with the least, and the highest among those with the greatest, reduction of androgens. A similar tendency can be observed with regard to the germinal epithelium.

344
Table 1.
Tabulation of the histologic findings and evaluation of the Leydig cell function on the basis thereof.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age, years</th>
<th>Weight of both testes together</th>
<th>Germinal epithelium</th>
<th>Sertoli cells</th>
<th>Capsule of the tubules</th>
<th>Relative number of Leydig cells</th>
<th>&quot;Absolute&quot; number of Leydig cells</th>
<th>Type of Leydig cells</th>
<th>Crystalloids of Reinke</th>
<th>Estimated function of Leydig cells</th>
<th>Testicular androgens according to Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>T</td>
<td>1-2</td>
<td>0-1</td>
<td>+</td>
<td>+</td>
<td>-0-1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0-1</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>19</td>
<td>0</td>
<td>+</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>30</td>
<td>1</td>
<td>++</td>
<td>1-2</td>
<td>1</td>
<td>0-1</td>
<td>+</td>
<td>+</td>
<td>2-3</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>69</td>
<td>0</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>72</td>
<td>0</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>74</td>
<td>1</td>
<td>++</td>
<td>2</td>
<td>1-2</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>82</td>
<td>3-4</td>
<td>++</td>
<td>0-1</td>
<td>4</td>
<td>3-4</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>+0.8</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>91</td>
<td>43</td>
<td>++</td>
<td>2-3</td>
<td>3</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>+0.9</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>66</td>
<td>2-3</td>
<td>++</td>
<td>1-2</td>
<td>3</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>18</td>
<td>67</td>
<td>2-3</td>
<td>++</td>
<td>1</td>
<td>1-2</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>2-3</td>
<td>1.0</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>74</td>
<td>2</td>
<td>++</td>
<td>2</td>
<td>1-2</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>18</td>
<td>75</td>
<td>3</td>
<td>++</td>
<td>1</td>
<td>4</td>
<td>3-4</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>+0.7</td>
</tr>
<tr>
<td>14</td>
<td>18</td>
<td>68</td>
<td>2</td>
<td>++</td>
<td>1-2</td>
<td>5-6</td>
<td>2-3</td>
<td>+</td>
<td>+</td>
<td>4-6</td>
<td>1.4</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>64</td>
<td>4</td>
<td>++</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>1.8</td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>73</td>
<td>2</td>
<td>++</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>2.2</td>
</tr>
<tr>
<td>17</td>
<td>18</td>
<td>59</td>
<td>3-4</td>
<td>++</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>2-3</td>
<td>2.7</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
<td>70</td>
<td>3</td>
<td>++</td>
<td>1</td>
<td>4</td>
<td>4-5</td>
<td>+</td>
<td>+</td>
<td>3-4</td>
<td>3.4</td>
</tr>
<tr>
<td>19</td>
<td>18</td>
<td>65</td>
<td>4</td>
<td>++</td>
<td>0-1</td>
<td>2-3</td>
<td>4</td>
<td>+</td>
<td>+</td>
<td>4-6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Explanatory notes:

Germinal epithelium: 0 = no epithelium; 1 = severely damaged epithelium; 2 and 3 = moderately and slightly damaged epithelium; 4 = normal appearance.

Sertoli cells: 0 = no cells; + = reduced number; ++ = ordinary number.

Capsule of the tubules: 0 = no thickening of wall; 1-3 = mounting degree of thickening; T = totally obliterated tubules.

Number of Leydig cells: 0-6 denotes rising number (4-5 commonest between ages of 20 and 50; 3-4 in men of over 50).

Reinke crystalloids: 0 = no crystalloids; 1-4 denotes rising number.

Estimated function: 0 = no function: + = function present but rather poor; ++ = fairly good function.

The mean weight of testicles was determined in 64 testicles, fixed in Stieve's fluid, from patients of over 60 whose testes were of normal or almost normal appearance. The mean weight was 40 gm. per pair of testicles.
Table 2.
Excretion of total 17-ketosteroid and androsterone and etiocholanolone before and after orchidectomy.

<table>
<thead>
<tr>
<th>Case</th>
<th>Before castration</th>
<th></th>
<th>After castration</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total 17-KS excretion mg./24 hrs.</td>
<td>Androsterone etiocholanolone excretion mg./24 hrs.</td>
<td>Androsterone etiocholanolone excretion mg./24 hrs.</td>
<td>Androsterone etiocholanolone reduction by castration</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.7</td>
<td>0.9</td>
<td>2.3</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>0.7</td>
<td>1.1</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>1.6</td>
<td>0.7</td>
<td>1.3</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>0.5</td>
<td>0.8</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>1.0</td>
<td>1.7</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>2.2</td>
<td>1.0</td>
<td>1.7</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>2.0</td>
<td>1.1</td>
<td>1.0</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>3.6</td>
<td>2.3</td>
<td>3.0</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>6.4</td>
<td>3.4</td>
<td>6.0</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>10</td>
<td>1.8</td>
<td>1.1</td>
<td>0.6</td>
<td>0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>11</td>
<td>4.2</td>
<td>2.4</td>
<td>2.9</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>12</td>
<td>2.9</td>
<td>1.6</td>
<td>1.5</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>3.8</td>
<td>2.1</td>
<td>1.9</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>3.5</td>
<td>2.2</td>
<td>1.6</td>
<td>0.8</td>
<td>1.4</td>
</tr>
<tr>
<td>15</td>
<td>4.5</td>
<td>2.3</td>
<td>1.4</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>16</td>
<td>5.3</td>
<td>3.4</td>
<td>2.4</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>17</td>
<td>5.8</td>
<td>4.3</td>
<td>2.8</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>18</td>
<td>7.7</td>
<td>5.3</td>
<td>3.3</td>
<td>1.9</td>
<td>3.4</td>
</tr>
<tr>
<td>19</td>
<td>6.4</td>
<td>4.3</td>
<td>1.7</td>
<td>0.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

The Sertoli cells have no apparent relation to the androgen production. The same applies to the thickness of the tubular capsule.

It will be seen from the table that a larger number of Leydig cells coincided with a greater reduction of androgens. Both the relative and the absolute number of cells appears to afford a good idea of the androgen production. Only in two cases (nos. 3 and 8) was the correlation poor. In each of these the histological examination suggested a greater function than was indicated by the androgen determinations.

In case 3 the Leydig cells were collected in small to medium-sized clumps. The cells themselves were medium-sized and mostly polygonal; a small number were elongated and resembled those in the connective tissue.

In the medium-sized cells the cytoplasm was in most cases finely and more or less homogeneously granulated, often containing crystalloids which were
sometimes clumped together. Other cells had a somewhat more irregular cytoplasm, with occasional pigment granules and occasional small vacuoles. There were no cells with any distinct ectoplasmic and endoplasmic zones. A few elongated cells were filled with pigment granules.

The nuclei were round, and virtually all of them had nucleoli. Anuclear cross sections were not more frequent than usual.

In case 8 most of the cells were of types B and C. A few elongated cells packed with pigment did not alter the impression of a good Leydig cell function in relation to the patient's age.

The possibility of errors in the urinary volumes in these two cases was naturally considered; but any errors of this kind could scarcely have been of such magnitude as to explain the discrepancy observed, and in any case the inclusion of three 24-hour volumes in each determination probably sufficed to eliminate them.

In this series the Reinke crystalloid content of the Leydig cells appeared to be well correlated with the androgen production.

CONCLUSIONS

No definite correlation was demonstrable in these elderly patients between the testicular weight, spermatogenesis, and thickness of the tubular capsule on the one hand, and the androgen production of the testicles on the other. Most of the patients nevertheless showed a correlation between this production and the »absolute« number of Leydig cells. The closest correlation was found, however, between the testicular production of androgens and the functional determination based on both the »absolute« number and the morphology of the Leydig cells. In the two patients where this was not the case, our investigation failed to explain the discrepancy.

Cell type A is mostly found in testicles with the poorest function, while type E occurs in testes with varying degrees of steroid production.

Of cell types B and C – which, according to Sniffen, have the best function – type B was the commonest in the present series, type C being found only in six cases, all of which had a relatively high steroid production. We subscribe to Sniffen's view that in normal testicles type B is generally more common than type C; moreover, in our experience type C is more common in the younger than in the older age groups.

Type D occurs, for natural reasons, fairly parallel with types B and C; it was found in most of the functioning testicles in this series.

Leydig cells of types B, C and D as well as Reinke crystalloids were relatively numerous in androgen-producing testicles. This suggests that the androgen production is confined to the Leydig cells, and that the above mentioned types represent cells with a secretory activity.
SUMMARY

In 19 patients with prostatic carcinoma the urinary content of androsterone and etiocholanolone was determined before and after castration. The value thus obtained for the androgen production of the testicles was correlated with the number and morphology of the Leydig cells, as evaluated in histological specimens. A fairly close correlation was found in all save two cases. In the two exceptions no cell details were observed which might explain the discrepancy.

REFERENCES

Bennett, H. S.: Am. J. Anat. 67, 151, 1940.
Benoit: Compt. rend. Soc. de biol. 87, 1922 (cited by Lundgren).
Romeis, B.: München. med. Wchnschr. 600, 1921.