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A CLINICAL AND HISTOCHEMICAL STUDY OF DISORDERS OF THE HUMAN TESTES

By

ABSTRACT

The testes of 50 subfertile and infertile males were investigated by clinical and histochemical methods. The enzyme activity per cell of interstitial cells of normal and abnormal testes was about the same. The output of the gonadotrophins of men with seriously damaged testes may be increased; the output of the oestrogens was normal and the output of 17-ketosteroids was normal or slightly decreased. Some of these patients showed a poor development of growth of the beard. The Sertoli cell is very resistant to atrophy and keeps a high enzyme activity for a long time. Besides an inhibiting effect of the steroids produced in the interstitial cells, it is also possible that under normal circumstances an inhibiting substance is produced in the Sertoli cells, which is necessary for a harmonious balance between hypophysis and testes.

The pathogenesis of morphological disorders of testes of men with insufficient semen are poorly understood. Several factors are known to be responsible for the development of these disorders (Sniffen et al. 1951; Sniffen 1952; Tonutti et al. 1960; Davis et al. 1965). Probably gonadal disorders occur more frequently because of primary disorders of the testes than because of abnormalities of the regulating hypothalamic-hypophyseal system. Disorders of different origin can lead to a similar morphological end-result.

In order to study some aspects of this problem the testes of 50 sub- and infertile men were investigated by clinical and histochemical methods.
The material comprised 50 men, who complained of sterility. The average age of the patients was about 30 years. Attention was paid among others to occupation, congenital disorders, cryptorchism, orchitis, surgical procedures in the neighbourhood of the genitals, trauma and varicocele. The volume of the testes was determined by the method of Schönfeld & Beebe (1942). These authors consider a total volume from 24 to 50 ml as normal, from 16 to 24 ml subnormal and less than 16 ml abnormally small. Furthermore the hair development and the physical habitus were recorded. At least two ejaculates from each patient were investigated. The fertility rate of an ejaculate was based on the density, motility and the morphology of the spermatozoa, according to the criteria of MacLeod & Gold (1956). Total gonadotrophins, oestrogens, 17-ketosteroids and 17-hydroxy cortisolsteroids were determined in the urine of the patients. The methods and the normal values have been described in the preceding paper (Koudstaal et al. 1967). A piece of tissue taken from the most normal testis was excised under local anaesthesia. The histology and the histochemical pattern of the normal testes have been described previously (Koudstaal et al. 1967). After the publication of Goldberg et al. (1964), who stressed the importance of the substrate androst-4-ene-3β,17β-diol for the demonstration of the enzyme 3β ol-hydroxysteroid dehydrogenase we investigated this reaction in the last 14 of our 50 patients. Fortunately these were spread over all four groups, in which the patients were divided (Table 1).

RESULTS

From every testis the degree of morphological abnormality was recorded after evaluation of the paraffin sections. The various criteria are indicated in Fig. 1. Generally various disorders were mixed up. Hence it was usually not possible to divide the patients into various definite groups. The general impression of the testis was used to place each patient in one of the groups to be described. The most important clinical and morphological findings are summarized in Table 1. There are no cases showing a decrease in the number of Leydig cells.

**Group I. Testes with defective spermatozoa only**

Clinically the two patients classified in this group showed no remarkable abnormality. The total volume of the testes of each man was respectively 36 and 40 ml. Density and motility of the spermatozoa in the ejaculate were normal (60–108 million/ml; 40–70 % good motility). The dry semen smear showed only defective spermatozoa, characterized by the absence of the head cytoplasm (Fig. 2). These cells were also seen in the testes (Fig. 3). No other morphological or histochemical abnormalities were found.

**Group II. Testes with mixed disorders**

This group was divided into two subgroups.

Subgroup A consisted of 22 patients with various moderate disorders of the testes (Fig. 4). As well as a varying grade of hypospermatogenesis and
### Table 1. Clinical and morphological findings.

<table>
<thead>
<tr>
<th>Pathology of the testes</th>
<th>Number of patients</th>
<th>Hair development</th>
<th>Testes volume acc. to Schönfeld &amp; Beebe (1942)</th>
<th>Sertoli cells</th>
<th>Cells of Leydig</th>
<th>Oligozoospermia</th>
<th>17-ketosteroids</th>
<th>17-hydroxy steroids</th>
<th>Oestrogens</th>
<th>Gonadotrophins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Normal</td>
<td>Abnormally small</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I. Only defective spermatozoa</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>n</td>
<td>n</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>II. Mixed disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Moderate</td>
<td>22</td>
<td>22</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>&lt;</td>
<td>&gt;</td>
<td>6</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>B. Serious</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>&lt;=</td>
<td>=&gt;</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>III. Germinal cell arrest</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>n</td>
<td>n/&gt;,</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>IV. Germinal cell aplasia</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Without or with slight fibrosis</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>n</td>
<td>&gt;</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>B. With moderate or severe fibrosis</td>
<td>9</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>&lt;=</td>
<td>=&gt;</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

N = normal; > = increased; < = decreased.

As can be seen by the figures not all data could be obtained from all patients. Details of the different groups are given in the text.
Morphological disorders of the human testis

Fig. 1.
Morphological abnormalities of primary gonadal disorders.

Fig. 2.
Phase contrast picture of dry semen smear. 665 X. Spermatozoa without head cytoplasmic. The semen of this patient contained only these cells. No other abnormalities were found.

germin al cell arrest a varying thickening of the wall of the seminiferous tubules was found. Generally a slight increase of the interstitial cells was registered as compared with the quantity of the tubules. The total volume of
the testes of each man varied from 16 to 38 ml. Most patients of this group had a small quantity of spermatozoa in the ejaculate. There were also some cases with azoospermia; probably these patients also had an obstruction of the epididymis of/and the ductus deferens. Two patients in this group had an orchitis at puberty. Another patient had undergone herniorraphy; one patient showed an unilateral cryptorchism and two patients had a large varicocele. One patient showed a slight increase in the output of 17-hydroxysteroids, namely 22.8 mg/24 h in the urine. The output of oestrogens in one patient was high: 27 µg/24 h. Two men had an increased output of gonadotrophins of respectively 0.40 and 0.84 mg/st/24 h. The other determinations showed nothing abnormal.

The 5 patients of subgroup B had seriously damaged testes. Only traces of spermatogenesis were found in all cases. Between the atrophic, hyalinized seminiferous tubules large clusters of interstitial cells were found (Fig. 5). The total volume of the testes of each male varied from 4 to 19 ml. One patient had some spermatozoa in the ejaculate, all the others showed azoospermia. Two patients had a slightly decreased output of 17-ketosteroids in the urine, namely 8.8 and 6.0 mg/24 h. One patient showed an increase of these meta-

Fig. 3.
T 216686. Paraffin section. H.E.-stain. 330 X. No normal spermatozoa can be seen. Normal testes volume. The same data as the patient of Fig. 2.
bolites (37.3 mg/24 h). In contrast to all the other patients this was combined with an increase in the 17-hydroxysteroids to 19.2 mg/24 h, along with an output of gonadotrophins of 0.50 mg per 24 h. This patient shaved himself very infrequently. Increased values of the gonadotrophins from two other men were 1.0 and 1.4 mg st/24 h.

The interstitial cells contained all the enzymes found in the interstitial cells of normal testes. The large, vacuolated Leydig cells contained many lipids. The activity of the enzyme acid phosphatase seemed to be slightly increased. Sometimes the activity of the non-specific esterases was increased. Both the compact and the vacuolated Leydig cells showed the presence of 3β ol-hydroxysteroid dehydrogenase (Fig. 5). Nearly all interstitial cells showed a very marked activity of the enzyme secondary alcohol dehydrogenase (Fig. 6). Normal seminiferous tubules between the more or less atrophic tubules, showed a normal enzyme pattern. Disappearance of spermatogenesis was coupled with lack of the enzyme alkaline phosphatase. Normally this activity is found in the spermatogonia and the spermatocytes. The resting, vital Sertoli cells showed an increase in enzymes acid phosphatase and 5-nucleotidase (Fig. 7) and a
high activity of the NADP- and NADPH-tetrazolium reductases. Sometimes the quantity of glycogen and of lipids was increased. Hyalinization of the tunica propria was coupled with a loss of all enzyme activity. Atrophy of the Sertoli cells was followed by a decrease of all enzymes. Activity of the enzymes acid phosphatase and 5-nucleotidase remains for a very long period during the atrophic process. Hence it is clear that the testes with mixed disorders show very divergent pictures when investigated by the several enzyme histochemical methods.

**Group III. Germinal cell arrest**

Although some other abnormalities of the testes of the 6 patients in this group were noted, the dominant feature was germinal cell arrest. In most of the cases the arrest was located at the level of the primary spermatocytes (Fig. 8). The total volume of the testes of each man varied from 17 to 44 ml. The output of the hormones from one patient is not known. The patient with the smallest testes had an increased output of total gonadotrophins in the urine (0.86 mg st/24 h). One patient had unilateral cryptorchism. No further
Fig. 6.
T 212229. Cryostat section. Secondary alcohohydrogease. 130 X. The same data as the patient of Fig. 5. All interstitial cells show a strong enzyme activity.

anamnestic or clinical abnormalities were recorded. The tubules which had only germinal cell arrest showed no remarkable enzyme histochemical differences as compared with that of normal seminiferous tubules.

**Group IV. Germinal cell aplasia**

This group was also subdivided in two subgroups (A and B). All patients lacked spermatogenesis. Patients of subgroup A had testes with seminiferous tubules without undue thickening of the wall (Fig. 9). The total volume of the testes varied from 16 to 26 ml. The interstitial cells seemed to be slightly increased. The lining of the tubules consisted of Sertoli cells only. One patient had an increased output of gonadotrophins in the urine (1.0 mg st/24 h) and another patient had a decreased output of the 17-ketosteroids (8.6 mg/24 h).

The testes of the 9 patients of subgroup B were composed of partly hyalinized tubules. At the same time the number of the interstitial cells was clearly increased. The volume of the testes ranged between 2 and 20 ml. Five patients of this group shaved themselves very infrequently. The urinary hormones were not determined in two patients. Five patients showed an increase in the total gonadotrophins and these patients had a normal or slightly de-
creased output of the 17-ketosteroids. The values of the increased gonadotrophins were respectively 0.40, 0.43, 0.48, 0.58 and 1.1 mg st/24 h. The decreased values of the 17-ketosteroids were respectively 6.5, 9.0, 9.2 and 9.3 mg/24 h. All patients in this group IV had azoospermia. There was nothing remarkable in the history. The histochemical abnormalities were the same as described in group II. In contrast to Jirásek & Rabuch (1963) we could not demonstrate any alkaline phosphatase in the Sertoli cells.

**DISCUSSION**

In our material a decrease in spermatogenesis was usually correlated with a relative increase in the number of interstitial cells. Atrophy of the tubules is mainly responsible for this phenomenon. On the other hand, the clusters of interstitial cells of some testes are so large, when compared with the volume of the testes, that the possibility cannot be excluded that the increase in the most seriously damaged testes (Table I, group II B and IV B) is also absolute. The enzyme histochemical data showed that the Leydig cells of abnormal
Fig. 8.


testes are capable of synthesizing 3-ketosteroids including probably androstenedione. It can be assumed that the total production of androstenedione by the various testes cannot greatly diverge. The output in the urine of the oestrogens of all patients was normal. The patients with seriously damaged testes showed a slight decrease in the output of the 17-ketosteroids. These findings confirm the observations of Maddock et al. (1952) and Sniffen et al. (1951). Whether androstenedione is converted to oestrogens or androgens cannot, as yet, be determined by enzyme histochemical methods. Quantitative biochemical data of abnormal testes are required to understand the slight discrepancy between the morphological and histochemical data and the output of the steroids in the urine. There is a complicated feed-back mechanism between the hypophyseal hypothalamic system and the testes (Albert 1961, Fig. 10). It is assumed that the testis produces an inhibiting factor which regulates the secretion of the gonadotrophins. The suggestion of Johnsen (1964) that an inhibiting substance is formed in the spermatids could not be confirmed since all cases of germinal cell arrest, except one in which the tubules were also atrophic, showed a normal output of gonadotrophins. The possibility that the
Sertoli cells produce an inhibiting factor could not be excluded since most patients with germinal cell aplasia without any atrophy of the Sertoli cells (group IV, A) showed a normal output of total gonadotrophins. The Sertoli cells are very resistant to atrophy and they show a very high enzyme activity.

On the other hand it is known that the hypophysis can be inhibited by steroids (Davidson & Sawyer 1961; Segal & Nelson 1959). Whether the increased secretion of gonadotrophins plays a part in the progression of the testicular disorders is an unresolved problem. Further work is needed to solve the many problems of the disordered feed-back mechanism between the hypophyseal-hypothalamic system and the testes of subfertile and infertile men.

ACKNOWLEDGEMENTS

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Fig. 10.
Hormonal control mechanism of spermatogenesis.

REFERENCES


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