URINARY TESTOSTERONE EXCRETION
IN MEN IN NORMAL AND PATHOLOGICAL CONDITIONS

By
A. A. A. Ismail and R. A. Harkness*

ABSTRACT

The excretion of testosterone in the urine of men has been studied using the method of Ismail & Harkness (1966 a); the estimates obtained with this procedure, which has satisfactory reliability criteria, agree with those from the majority of published methods. The effect of time, age, sexual activity, adrenal hyperfunction, hypopituitarism, undernutrition, precocious puberty, oligospermia and chlorpromazine administration have been investigated in a small number of subjects. All the above factors, except in the present case of oligospermia, are associated with alterations in testosterone excretion. The possible mechanisms and significance of these changes are discussed.

In the investigation of gonadal function in men, urinary 17-oxosteroid determinations have not proved of great value because the testis contributes only about one third of the total urinary 17-oxosteroid excretion, the remaining two thirds arising from precursors excreted by the adrenal cortex (Vander Wiele et al. 1963). The wide range of variation in 17-oxosteroid excretion in normal males, from 5 to 15 mg/24 h mainly reflects variations in secretion of precursors by the adrenal cortex. However, the estimation of testosterone in urine provides a satisfactory index of total testosterone production which in the normal male is largely due to testicular secretion of the hormone (Vander Wiele et al. 1963; Kirschner et al. 1965; Horton et al. 1965). Only small quantities of urinary testosterone arise from precursors secreted by other sources (Vander Wiele et al. 1963; Camacho & Migeon 1964). Because of the

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small extra-gonadal production of testosterone in men, variations in this component have not been sufficient to explain the large range of urinary testosterone levels found by previous workers (see Ismail & Harkness 1966a).

The purpose of the present study was to assess possible causes of variations in testosterone excretion. Thus the effect of time, age, sexual activity, adrenal hyperfunction, hypopituitarism, undernutrition, precocious puberty, oligospermia, and chlorpromazine administration have been studied. Preliminary accounts of some of these findings have been published (Ismail & Harkness 1966b, c).

MATERIALS AND METHODS

Design of investigation

Six normal men ranging in age from 20–34 a were studied; each subject was normally active and in good health. All the subjects who were studied collected complete 24 h urine samples continuously throughout the period of investigation starting at 8–9 a.m. except in subjects H and J who started at about 12 in the morning; subject H altered his regime on day 8 starting the collections at 12 midnight. The effect of sexual activity on testosterone excretion was investigated on two subjects E and NC; no sexual activity took place before the investigation started for a period of 18 and 7 weeks for subject E and NC respectively. Sexual activities restarted during the period of investigation. Urines were analysed as single 24 h samples in order to detect any rapid changes in hormone output or as 2 to 10 day urine pools when mean levels were required.

Assay methods

For the estimation of 17-hydroxycorticosteroids (17-OHCS) and total 17-oxosteroids, modifications of the techniques described by Appleby et al. (1955) and Vestergaard (1951) were employed. Urinary testosterone excretion was estimated by the method of Ismail & Harkness (1966a). Moreover, the studies on subjects H and J were performed after the addition of 4-\(^{14}\)C testosterone as an internal standard to correct for losses during the procedure. All samples were assayed after a period of storage at 4 °C of less than 2 weeks. In order to investigate the effect of storage of urine at 4 °C, three samples were kept for 10 weeks and the assays repeated. The results were 60–80 % lower.

RESULTS

In general, the results obtained with the present method are similar to earlier estimates for normal men but the maximal values are lower than those obtained with some of the previous methods. In an initial series of studies the mean value for men between the ages of 21 and 63 was 51.7 μg/24 h (see Ismail & Harkness 1966a).
Day to day variation in the testosterone excretion of normal men

Day to day variation in urinary testosterone was studied in 5 subjects for periods of 13 to 29 days; marked alterations were found with peaks occurring at regular intervals.

The results on subject E showed marked variations in the levels of urinary testosterone (Fig. 1). Moreover these variations appeared to be cyclic with peaks at about 12 days intervals. It should be noted that the results shown in Fig. 1 were obtained using enzymic hydrolysis. The other studies were performed using acid hydrolysis; the results from these two methods showed little or no difference. The study on subject ED showed a similar variation in testosterone excretion from 40.3 to 90.5 μg/24 h with peaks at about 6 day

Fig. 1.
Excretion of testosterone, 17-oxosteroids and 17-hydroxycorticosteroids by Subject E.
intervals (Fig. 2). A further study on subject NC also showed considerable variation in testosterone excretion from 44.1 to 63.5 μg/24 h (Fig. 3); peaks of excretion occurred at intervals of about 5 days. There is no marked synchronisation of testosterone peaks with those of 17-oxosteroids or of 17-hydroxycorticosteroids in these three studies except on day 13 in Fig. 2.

Testosterone levels in two other subjects H and J (Table 1), both aged 20, also showed variations. In subject H from 39.2 to 113.3 μg/24 h with peaks at 5-6 day intervals and in subject J from 61.4 to 149. μg/24 h with peaks at 4 to 6 day intervals. Both men were studied for a period of 17 days and the results were corrected by the recoveries obtained using 4-14C-testosterone as an internal standard, in this way the chance of any systematic cyclic variations in recoveries was reduced.

Other factors affecting testosterone excretion

Sexual activity

The effect of sexual activity on testosterone excretion was studied on subjects E and NC (Figs. 4 and 5). There were marked variations in the urinary testosterone excretion in both subjects, with regular peaks at about 8 day intervals in subject E and at 5 day intervals in subject NC throughout the period.

![Graph showing testosterone, 17-oxosteroids, and 17-hydroxycorticosteroids excretion by Subject ED.](image-url)
Fig. 3.
Excretion of testosterone, 17-oxosteroids and 17-hydroxycorticosteroids by Subject NC.

Table 1.
Day to day variation of testosterone levels in the urine of normal men.

<table>
<thead>
<tr>
<th>Day</th>
<th>Testosterone μg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject H</td>
</tr>
<tr>
<td>1</td>
<td>74.2</td>
</tr>
<tr>
<td>2</td>
<td>79.7</td>
</tr>
<tr>
<td>3</td>
<td>79.8</td>
</tr>
<tr>
<td>4</td>
<td>85.9</td>
</tr>
<tr>
<td>5</td>
<td>70.7</td>
</tr>
<tr>
<td>6</td>
<td>74.4</td>
</tr>
<tr>
<td>7</td>
<td>68.5</td>
</tr>
<tr>
<td>8</td>
<td>79.5</td>
</tr>
<tr>
<td>9</td>
<td>92.5</td>
</tr>
<tr>
<td>10</td>
<td>54.6</td>
</tr>
<tr>
<td>11</td>
<td>39.2</td>
</tr>
<tr>
<td>12</td>
<td>71.3</td>
</tr>
<tr>
<td>13</td>
<td>108.2</td>
</tr>
<tr>
<td>14</td>
<td>113.3</td>
</tr>
<tr>
<td>15</td>
<td>96.1</td>
</tr>
<tr>
<td>16</td>
<td>85.4</td>
</tr>
<tr>
<td>17</td>
<td>79.3</td>
</tr>
</tbody>
</table>

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Excretion of testosterone, 17-oxosteroids and 17-hydroxycorticosteroids by Subject E.

- Denotes sexual activity.

of investigation. However, the levels when these men were not sexually active were generally lower than those observed when the normal sexual activities restarted (Figs. 4 and 5). It should be noted that there is a reasonable correlation between 17-oxosteroid excretion, in subject E, with that of urinary testosterone. The mean levels of urinary testosterone when subject E and NC were not sexually active were 42 and 37 μg/24 h respectively; when sexual activities were started the mean levels rose to 55.0 and 66 μg/24 h respectively.

Removal or altered function of endocrine organs

In the present study, the excretion of testosterone by two castrate men was similar to that of amenorrhoic women. No marked fluctuation was observed in the levels of the castrate men during the 12 day period; these levels are in agreement with those obtained by Futterweit et al. (1964) and Gibree et al. (1965). Further evidence that the testis excretes the majority of the precursors
of urinary testosterone is provided by the normal urinary levels of the hormone in two adrenalectomized men. The low levels of the hormone in hypopituitarism show that the pituitary gland is necessary for the maintenance of normal levels of urinary testosterone (see Table 2). It should also be noted from the results in Table 2 that a deficiency in the tubular function of the testis is not necessarily associated with abnormalities in the endocrine function. Although the contribution of the adrenal cortex to urinary testosterone levels is normally small compared to that from the testis, this contribution can be large when there are tumours of the adrenal cortex.

Fig. 5.
Excretion of testosterone, 17-oxosteroids and 17-hydroxycorticosteroids by Subject NC.

- Denotes sexual activity.
Table 2.
Mean urinary testosterone levels in men.

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Age in years</th>
<th>Duration of urine collection in days</th>
<th>Testosterone in μg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrate</td>
<td>57</td>
<td>12</td>
<td>4.8</td>
</tr>
<tr>
<td>Castrate</td>
<td>52</td>
<td>12</td>
<td>2.7</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>60</td>
<td>2</td>
<td>38.4</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>22</td>
<td>12</td>
<td>41.1</td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td>28</td>
<td>14</td>
<td>5.9</td>
</tr>
<tr>
<td>Oligospermia</td>
<td>30</td>
<td>7</td>
<td>43.3</td>
</tr>
<tr>
<td>Adrenal cortical carcinoma</td>
<td>51</td>
<td>2</td>
<td>323.7</td>
</tr>
<tr>
<td>Psychogenic undernutrition</td>
<td>17</td>
<td>10</td>
<td>6.2</td>
</tr>
<tr>
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<td>17</td>
<td>10</td>
<td>11.2</td>
</tr>
<tr>
<td>Psychogenic undernutrition</td>
<td>20</td>
<td>10</td>
<td>31.2</td>
</tr>
<tr>
<td>TB and undernutrition</td>
<td>67</td>
<td>2</td>
<td>30.5</td>
</tr>
<tr>
<td>Gastro-intestinal disease and undernutrition</td>
<td>40</td>
<td>11</td>
<td>6.5</td>
</tr>
<tr>
<td>Chlorpromazine treatment</td>
<td>32</td>
<td>2</td>
<td>21.4</td>
</tr>
<tr>
<td>Chlorpromazine treatment</td>
<td>36</td>
<td>2</td>
<td>23.3</td>
</tr>
<tr>
<td>Chlorpromazine treatment</td>
<td>36</td>
<td>2</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Undernutrition and Chlorpromazine

The effect of undernutrition associated with psychological and physical diseases was investigated. Three young men aged 17–20 with severe undernutrition associated with psychological disturbances had low urinary testosterone levels. Although all 3 subjects had diminished sexual urges, this reduction was less marked with the subject with a level of 31.2 μg/24 h.

Depression of the endocrine functions of the testis was also associated with the administration of chlorpromazine; the mean excretion of urinary testosterone in three men aged 20–36 years treated with chlorpromazine was low.

Age

The effect of age upon urinary testosterone levels is a problem which is at present under investigation. In the present study the testosterone excretion of men aged between 21 and 40 was approximately 40 to 80 μg/24 h. Men over 50 years have tended to show somewhat lower levels. The levels in normal young men aged 15–20 years have ranged from about 20 to 100 μg/24 h. In the present investigation two cases of precocious puberty have been studied aged 4 and 3 years, the levels were 7.3 and 12.5 μg/24 h respectively.
DISCUSSION

Testosterone is the most potent androgen known to be present in human blood. Its measurement thus provides an estimate of the androgenic stimulation to which the body is exposed. In man, the production rates based on plasma levels and on those based on urinary excretion are similar (Horton et al. 1965; Rivarola et al. 1966). Thus the urinary levels of the hormone provide an index of the testicular secretion secretion of androgen. The demonstration of regular peaks in testosterone excretion in the present study suggests, but certainly does not prove, that there may be a cyclic variation in the testicular secretion of the hormone. The present findings are consistent with the results of Exley & Corker (1965) who demonstrated a cyclic alteration in 17-oxosteroid and oestrone levels which was not associated with a similar change in urinary 17-OHCS in normal men. A detailed mathematical analysis of changes in 17-oxosteroid excretion has been reported by Halberg et al. (1965) who demonstrated a weekly cycle in the 17-oxosteroid levels in a normal man. However, in the present investigation, variations in testosterone excretion have not generally been correlated with alterations of 17-oxosteroid output which tended to be synchronized with the urinary 17-OHCS arising from compounds secreted by the adrenal cortex. However, there may be some correlation between all 3 parameters in the first peaks of the studies shown in Fig. 4.

The importance of the gonadal hormones in maintaining sexual drives and activity has long been recognized (Young 1961) and this earlier evidence is consistent with the present findings of reduced sexual urges in undernourished young men. In the present study, the relationship between sexual activity and testosterone excretion was investigated. The results showed that sexual activity had little effect on the occurrence of regular peaks of testosterone excretion. However, an increase in the level of testosterone excretion was found which paralleled sexual activity; the results in Fig. 5 are in agreement with the findings of Branton et al. (1952) as quoted by Mann (1964). Previous studies by Lindner & Mann (1960) have shown that the levels of fructose in semen are maintained by testosterone and that there is a positive correlation between the levels of fructose and citric acid in the seminal vesicles and the levels of circulating testosterone in animals. Thus the findings are in agreement with those of previous investigators using other techniques. Moreover, the maturation of spermatozoa in the genital tract is indirectly dependent upon the presence of testosterone (Bishop 1961; Dawson et al. 1957; Dawson & Rowlands 1959; Maraud & Stoll 1958). From the results of the present investigations, the partial emptying of the tract due to sexual activity may be associated with a stimulation of testicular secretion. Such an increase in hormone levels might increase secretion in the male genital tract and thus accelerate the refilling of the system.
In the present investigation the normal level of urinary excretion of testosterone by adrenalectomized men and the low levels in two castrate men confirmed that the contribution of the adrenal cortex to urinary testosterone is normally small compared to that from the testis (see Rosner & Conte 1966; Tamm et al. 1966a; and others). However, the high levels in patients with adrenocortical tumours are not an accurate indication of plasma testosterone levels. The amounts of testosterone, androstenedione, dehydroepiandrosterone and 17α-hydroxyprogesterone which are converted to urinary testosterone have been described by Camacho & Migeon (1964). The conversion of small amounts of epitestosterone to urinary testosterone has also been demonstrated (Tamm et al. 1966b). The difficulties of interpretation of urinary testosterone levels have been discussed in detail by Tait & Horton (1964).

The level of testosterone secretion is presumably controlled through the pituitary, possibly by the luteinizing hormone. Aakvaag et al. (1965) and others have shown that HCG, a hormone with LH-like activity, stimulates secretion by the testis and that human pituitary FSH has much less effect. Kirschner et al. (1965) have shown that synthetic androgens depress endogenous testosterone levels. These workers have suggested that circulating androgen levels are one controlling factor in the regulation of pituitary secretion. The very low level of urinary testosterone found in a subject with hypopituitarism in the present study was similar to that reported by Rosner et al. (1965). The results of the present investigation are also consistent with those of Futterweit et al. (1965). Thus the evidence of the present investigations and those of other workers are consistent with hypothalamic-pituitary regulation of testosterone secretion.

The results of the present studies show that undernutrition is associated with a marked reduction in testosterone excretion in some cases to castrate levels. Similar changes have been shown to occur in animals, generally by less direct methods; these changes may be mediated through the anterior pituitary gland. Moore & Samuels (1931) showed that inadequate nutrition led to regression in the male accessory sex organs which could be reversed by the administration of either testicular hormone or anterior pituitary extracts. Similar results were obtained by Mann et al. (1960) and by Setsell et al. (1965).

The effect of drugs upon testosterone excretion has also been studied. Marked stimulation of the endocrine function of the testis by the synthetic compound, clomiphene, has been demonstrated by Harkness et al. (1965). The low levels of urinary testosterone after chlorpromazine administration demonstrate another depressant effect for this drug which has been shown by Barraclough & Sawyer (1957) to block ovulation in the rat. This depressant action may be mainly due to its action on the central nervous system, however, an additional direct effect is also possible (Hopkin 1955).

In the present investigation although the levels in precocious puberty were higher than those reported by Zurbrugg et al. (1965) for normal children, they
are considerably lower than those of normal adult men. The difference may in part be due to the influence of body weight on total amounts excreted.

At present there is no general agreement on the age at which urinary testosterone levels are maximal. In a study of 59 males aged 12 to 82 years, Morel-Fargas & Nowakowski (1965) using single 24 h samples of urine, found mean testosterone excretion rose to a maximum at about the age of 25 years and fell thereafter. A steady increase in mean testosterone excretion between 10 and 19 years has also been reported by Knorr (1967). These increases are similar to the changes in the urinary 17-OHCS arising from adrenal cortex as described by Borth et al. (1957). However, in the present preliminary investigation and in those of Ibayashi et al. (1964) and Lim & Dingman (1965), higher maximum levels were found in the 15–20 years age period.

In general the results of investigating testosterone excretion in the urine of men over long periods suggest that there is a considerable variation in the secretion of the precursors of urinary testosterone by the testis. It may, therefore, be necessary to determine urinary testosterone for about 10 days in men, in order to detect the majority of abnormalities of testosterone excretion.

ACKNOWLEDGEMENTS

A. A. A. I. is indebted to the government of the United Arab Republic for financial support. The help of Professor R. B. Fisher and Dr. J. A. Loraine is gratefully acknowledged. We are also grateful for the help of Wm. S. Merrell Inc.

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Received on February 10th, 1967.