GONADOTROPIC ACTIVITY IN POOLED URINE WITH ACIDIFICATION*

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SYNOPSIS

Since a gonadotropin-inhibiting substance in human urine is precipitated by acidification of urine, comparisons of the gonadotropic activities and the inhibitory effects in urine after acidification and removal of precipitate and in untreated urine were carried out in bioassays. Gonadotropic activity in the supernatant of acidified urine, in which a gonadotropin-inhibitor was removed through precipitation, was somewhat higher than that in non-acidified urine in the ovulation method. Inhibition on ovulation was obtained in the rats injected the heated extract taken from the non-acidified urine, while no inhibition was obtained in those given that taken from the supernatant of acidified urine. These findings suggest that there is a possibility that the assayed gonadotropic activity of urine without acidification by certain methods is slightly lower than the actual activity by the presence of the inhibitor.

The existence of a gonadotropin inhibiting substance in the extract, which was obtained from the precipitate of acidified urine of adult males by a borate buffer solution, has been reported (Ota et al., 1967 a, b, 1968b). Consequently, the supernatant of acidified urine from which precipitate was removed might have stronger activity of gonadotropin than untreated urine. In this paper, a comparison of gonadotropin activities of urine pooled with and without acetic acid is discussed using general bioassay methods for human pituitary gonadotropins and, furthermore, a comparison of the inhibitory effects of heated extracts from the urine specimens pooled with and without acetic acid is described.

MATERIALS AND METHODS

Urine was collected from 8 healthy adult males ranging from 27 to 38 years of age during their working hrs. and kept at 4—5°C without using preservatives for 1—4 days before it was processed. After mixing thoroughly, the pooled urine was divided equally into two portions. One half of the urine was not treated and the other half was acidified to pH 3.8—4.0 with glacial acetic acid and both portions were stored at 4—5°C for two days. A heavy precipitate which developed in the acidified urine was removed by centrifugation. Both the non-acidified urine and the supernatant of the acidified urine were extracted according to the tannic acid precipitation method of Johnsen (1958). Each of the residues, kept in a desiccator in a cold room, was dissolved in a 0.1 M sodium borate buffer solution (pH 8.6) prior to bioassay.

In the first experiment, each extract was injected subcutaneously into 21-day-old intact female rats of the Wistar strain, obtained from animal laboratory A, at 11.00 to 11.30 a.m. for 3 successive days in a daily dose of 0.05 l or 0.1 l equivalent urine per 0.3 ml of an injection volume. To the control group, 0.3 ml of a sodium borate buffer solution was injected instead of urine extract. The rats were killed at
Table 1. Comparison of stimulating-activities of urine pooled with and without acidification

<table>
<thead>
<tr>
<th>Urine used for extraction</th>
<th>Extract 1Eq*</th>
<th>Average dry weight of end-product (mg/l)</th>
<th>No. of rats</th>
<th>Initial body weight (Av.g±S.D.)</th>
<th>Final body weight (Av.g±S.D.)</th>
<th>Uterine weight (Av.mg±S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-acidified urine</td>
<td>0.15 l</td>
<td>58.1</td>
<td>12</td>
<td>40.9±1.4</td>
<td>50.0±2.1</td>
<td>37.8±5.9</td>
</tr>
<tr>
<td></td>
<td>0.30 l</td>
<td></td>
<td>12</td>
<td>41.0±1.3</td>
<td>49.8±3.6</td>
<td>81.3±6.7</td>
</tr>
<tr>
<td>Supernatant of acidified urine</td>
<td>0.15 l</td>
<td>33.1</td>
<td>12</td>
<td>40.9±1.5</td>
<td>49.4±2.4</td>
<td>38.1±2.4</td>
</tr>
<tr>
<td></td>
<td>0.30 l</td>
<td></td>
<td>12</td>
<td>41.0±1.2</td>
<td>49.8±3.7</td>
<td>83.4±3.7</td>
</tr>
</tbody>
</table>

* The extract obtained from 0.15 or 0.30 l of adult males' urine as a total dose was injected.

Table 2. Comparison of human pituitary gonadotropins in urine pooled with and without acidification

<table>
<thead>
<tr>
<th>Urine used for extraction</th>
<th>Average dry weight of end-product (mg/l)</th>
<th>No. of rats</th>
<th>Initial body weight (Av.g±S.D.)</th>
<th>Final body weight (Av.g±S.D.)</th>
<th>No. of ovulated rats</th>
<th>Percent of ovulation (%)</th>
<th>No. of ova per rat (Av. ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-acidified urine*</td>
<td>47.7</td>
<td>37</td>
<td>31.8±2.0</td>
<td>38.0±2.2</td>
<td>12</td>
<td>32.4</td>
<td>14.16±12.2</td>
</tr>
<tr>
<td>Supernatant of acidified urine*</td>
<td>28.8</td>
<td>37</td>
<td>31.9±2.1</td>
<td>39.0±2.1</td>
<td>20</td>
<td>54.1</td>
<td>23.26±14.0</td>
</tr>
</tbody>
</table>

* The extract obtained from 0.8 l of urine was divided into three portions and injected subcutaneously once a day for three days. ** Calculated by chi square test.

9.30 a.m. on the 4th day and the uteri were weighed.

In the second experiment, each extract was injected into 19—20 days old female rats of the Wistar strain, purchased from animal laboratory B, in a total dose of 0.8 l equivalent urine by the same manner as in the previous experiment. The rats were killed 20—21 hrs. after the final injection and the number of ova recovered from the oviducts was counted with the aid of a dissecting microscope and trans-illumination.

In the third experiment, the ovulation method was employed to detect the inhibitory effects of both extracts. As the control, 4 I.U. of pregnant mare's serum gonadotropin (PMS) was injected intraperitoneally into 19- to 20-day-old rats at 2 p.m. and 4 I.U. of human chorionic gonadotropin (HCG) was given subcutaneously 48 hrs. after the injection of PMS. In the experimental groups, each extract, equivalent to 0.8 l of urine, which was heated in a boiling water bath for one hr. to destroy human pituitary gonadotropins was given with the injection of HCG, because it has previously been shown that this inhibitor has an inhibitory effect mainly on luteinizing hormone (Futterweit et al., 1963; Ota et al., 1968 a). The dissection was performed 18—19 hrs. after the injection of HCG.

RESULTS

The weights of the uteri in the control and experimental groups received the extract equivalent to 0.15 l or 0.30 l of urine in a total volume are shown in Table 1. No differences were observed in the average weights of the uteri between the animals administered the extract obtained from supernatant of the acidified urine and those given the extract from the non-acidified urine.

As shown in Table 2, the average number of ovulated eggs per rat and the percentage of ovulation were higher in the rats administered the extract from the supernatant of
Table 3. Comparison of inhibition of heated extracts from acidified and non-acidified pooled urine specimens on ovulation

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Average dry weight of end-product (mg/l)</th>
<th>No. of rats</th>
<th>Initial body weight (Av.± S.D.)</th>
<th>Final body weight (Av.± S.D.)</th>
<th>No. of ovulated rats</th>
<th>No. of ova per rat (Av.± S.D.)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>12</td>
<td>36.0±3.0</td>
<td>45.9±3.0</td>
<td>12</td>
<td>12.48±3.0</td>
<td></td>
</tr>
<tr>
<td>Non-acidified urine*</td>
<td></td>
<td>12</td>
<td>36.3±2.8</td>
<td>46.0±2.9</td>
<td>10</td>
<td>6.71±4.9</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Supernatant of acidified urine*</td>
<td></td>
<td>12</td>
<td>36.5±2.8</td>
<td>46.8±3.0</td>
<td>11</td>
<td>12.28±4.7</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

* The heated extract from 0.8 l of the non-acidified or the supernatant of the acidified urine was injected with 4 I.U. of HCG, 48 hrs. after the injection of 4 I.U. of PMS.

The findings obtained in the present experiments propose that one who attempts to extract gonadotropins in unacidified urine should keep in mind that the inhibitor exists in the extracts, even though its inhibitory effect on certain bioassays is not significant. Furthermore, they propose the necessity of

**DISCUSSION**

A gonadotropin-inhibiting substance in human urine was first found by Landau et al. (1960) and has been further demonstrated by several authors (Soffer et al., 1961, 1962; Soffer and Fogel, 1963, 1964; Saito, 1965; Davis et al., 1966; Ota et al., 1968 a, 1968 b). Little information, however, has been available as to the influence of the inhibitor on the bioassay of urinary gonadotropins.

It has not been proved that the inhibitor is completely removed from the urine by acidification. However, a large portion of this substance is possibly precipitated in the acidified urine as indicated by the fact that no inhibition was obtained in the rats injected with heated extract from the supernatant of acidified urine.

In the present study, no difference was found between the uterine weight stimulating activities of the extracts from the supernatant of acidified urine and non-acidified urine. However, in the ovulation method, the gonadotropic activity in the extract from the supernatant of acidified urine was somewhat higher than that of non-acidified urine.

The uterine weight response method is a non-specific assay method for pituitary gonadotropins and is mainly based on the secretion of estrogen by the gonadotropin-stimulated ovaries. The ovulation method is relatively specific for HCG and luteinizing hormone (LH). Follicle stimulating hormone (FSH) predominates over LH in crude kaolin extracts of the urine of males. The action of the inhibiting substance is directed against HCG or LH, but not against FSH (Futterweit et al., 1963). These facts seem to explain the results which were obtained.

The findings obtained in the present experiments propose that one who attempts to extract gonadotropins in unacidified urine should keep in mind that the inhibitor exists in the extracts, even though its inhibitory effect on certain bioassays is not significant. Furthermore, they propose the necessity of
differential measurements of both the activity of gonadotropin and the inhibitor in urine.

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REFERENCES


