IMMUNOREACTIVE GROWTH HORMONE IN HUMAN URINE

By

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ABSTRACT

By using a double antibody radio-immunoassay (pre-precipitation technique) for the determination of immunoreactive human growth hormone (IRHGH) in normal human urine concentrated by dialysis and lyophilization, a factor was revealed that displaces $^{125}$I-HGH from HGH antibodies. This displacement was neither due to salts nor to glucose; it is suggested that it is due to IRHGH in the urine. A linear relationship between dilution of urine and the measured IRHGH concentration was obtained. Recovery of exogenous HGH was between 70–105%. The recovery of IRHGH from different volumes of urine following dialysis and lyophilization was between 97–110%.

Plasma IRHGH and urinary IRHGH was measured simultaneously after HGH injection in a normal subject. A correlation was shown between plasma IRHGH and urinary IRHGH. In 9 normal subjects, the urinary IRHGH ranged from 28–53 ng/24 h. The excretion of urinary IRHGH was increased in acromegaly and was diminished in some, but not in all patients with adult hypopituitarism.

The urinary IRHGH was further studied by gel filtration. It was recovered in one peak corresponding to a molecular weight of approximately 20,000–30,000. However, in the present work it was not clarified whether the urinary IRHGH represents pituitary HGH excreted in the urine or a metabolite of high molecular weight with retained immunological properties.

Immunoreactive human growth hormone (IRHGH) in the plasma fluctuates during the day and night (Quabbe et al. 1966) and in order to assess the daily production of IRHGH, it is necessary to draw frequent blood samples during both day and night and to know the metabolic clearance rate for IRHGH in the subject. Fraser & Wright (1968) expressed this difficulty in the following way: «With the variability of serum levels of GH found in all subjects except in acromegaly, it is difficult to assess the daily GH production by the pituitary. In such conditions as diabetes mellitus this may be an important factor and urinary measurements might provide a useful estimate of the mean rate of GH secretion. As yet a satisfactory concentrating procedure has to be evolved to enable the levels to be measured in normal subjects.»

Insulin determinations in human urine were relatively easily performed (Jørgensen 1966; Rubenstein et al. 1967). However, attempts to measure IRHGH in normal human urine have met several difficulties (Girard & Greenwood 1968; Glick 1968; Srivastava et al. 1971; Lowy et al. 1971). There are several reasons for this; firstly the concentration of IRHGH in normal unconcentrated human urine is very low indeed and well below the sensitivity of any radio-immunoassay method for IRHGH known at the present time. Furthermore, as Girard & Greenwood (1968) have pointed out, salts might mimic the effects of IRHGH in normal urine.

This study was undertaken to investigate whether a suitable concentrating procedure for human urine would reveal IRHGH in normal human urine and also whether the IRHGH in normal urine reflects plasma levels.

**MATERIAL AND METHODS**

Collection and concentration of urine. – The urine was voided directly into a plastic container from Statens Serum Institute, Copenhagen. In women a plastic funnel was used. During the collection period, the urine was stored at 4°C and then at –20°C if dialysis was not carried out at once. Approximately 0.5 g purified human albumin (Statens Serum Institute) was added to the plastic container before it was emptied in order to minimize the absorption of IRHGH by the container. Dialysis was performed at 4°C in Visking tubes 18/32 against re-distilled water. Dialysis went on for 36 hours and re-distilled water was renewed twice. In routine experiments, 50 ml urine were dialysed. The dialysed urine was lyophilized and dissolved in 1 ml 0.04 M phosphate buffer pH 7.4 containing 0.5% purified human albumin and 0.9% NaCl. pH was adjusted to 7.4 with 2 M NaOH. The concentration of NaCl was measured by flamephotometry of untreated urine and after the pH had been adjusted. The concentrated urine was centrifuged and the supernatant was pipetted off for the radio-immunoassay.

Radio-immunoassay. – 200 µl of the supernatant (see above) was pipetted off in duplicate for the radio-immunoassay of IRHGH according to Hanssen (1972). The concentrated urinary samples were substituted for the plasma samples in the method described. The variability of the assay is identical to that described for the assay in

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plasma (Hanssen 1972). The lower detection limit given in terms of the minimum amount IRHGH detectable per ml of original urine is better than 0.004 ng/ml.

**Gel filtration.** – Sephadex G-100 (Pharmacia, Uppsala, Sweden) was packed in a column 53 × 0.9 cm I. D. The column was eluted at room temperature with phosphate buffer 0.04 M pH 7.4 containing 0.9 NaCl and 1% purified human albumin. 1 ml urine concentrated from 400 ml according to the described procedure was applied to the column. The flow rate was 19 ml/h and 1 ml fractions were collected by means of an automatic fraction collector (LKB, Bromma, Sweden). The fractions were assayed in duplicate for IRHGH. To study the elution pattern, blue dextran, myoglobin and NaI were added to the column in a separate experiment, eluted in an identical way and O. D. measured.

**Normal subjects** consisted of 6 men aged 28–35 years and 3 women aged 22–34 years. All were within 10% of their ideal weight and showed no evidence of endocrine disorders.

**Acromegaly.** – All the patients had clinical and laboratory signs pointing to acromegaly. All but one had elevated plasma IRHGH as measured by the method of Hanssen (1972) (mean 130 ng/ml, range 4.2–704 ng/ml plasma).

**Adult hypopituitarism.** – 3 patients without HGH response to insulin induced hypoglycaemia as defined by Hanssen (1972) were studied (empty sella syndrome, cranio-pharyngeoma op., hypothalamic tumour).

**RESULTS**

**Evaluation of special factors that might influence radio-immunoassay in urine**

a. Fig. 1 demonstrates that increasing concentrations of NaCl affects the percentage of radioactivity precipitated. However, by means of the concentrating process described above, the urine contributed with not more than 100 mEq./l Na⁺ and usually between 50–80 mEq./l Na⁺. This will not influence the assay to more than a minor extent.

b. Glucose in concentrations up to 5% will not influence the radioactivity precipitated.

c. Urea: The addition of 3.6 mg urea to 0 and 0.78 ng/ml HGH standards did not alter the precipitated radioactivity.

The possible cross-reaction between precipitating antiserum and urine was studied by immuno-electrophoresis according to Brunfeldt & Jørgensen (1967). No cross-reaction was demonstrated.

**Investigation of the collection of urine**

a. Storage of urine at 4°C for three days did not affect the results of the radio-immunoassay.

b. Storage of urine for 24 hours at room temperature reduced the IRHGH demonstrated in urine: Urine + added HGH = 9.8 ng/ml. After 24 hours at 20°C = 6.3 ng/ml.
The influence of NaCl on precipitation

![Graph showing the effect of NaCl concentration on precipitation](image)

Fig. 1.
The effect of different concentrations of NaCl on the precipitated radioactivity.

c. Albumin (0.5 g/l) added to the urine container before the concentrating process did not significantly influence the precipitated radioactivity observed:
Without albumin 22.4 %
with albumin 24.5 %.

Investigation of the concentrating procedure

a. In Table 1 the recovery experiments with exogenous HGH added to urine or albumin buffer before the concentrating process are shown. These samples were then subjected to the concentrating process described. Recovery was between 70–105 %.

Table 1.
Recovery experiments. Exogenous HGH added to urine or to albumin buffer before the concentrating process. The usual concentrating process was then performed.

<table>
<thead>
<tr>
<th>Urine value ng/ml</th>
<th>Added HGH ng/ml</th>
<th>Measured ng/ml</th>
<th>Recovery ng/ml</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9</td>
<td>6.25</td>
<td>9.8</td>
<td>5.9</td>
<td>94</td>
</tr>
<tr>
<td>1.1</td>
<td>3.13</td>
<td>3.3</td>
<td>2.2</td>
<td>70</td>
</tr>
<tr>
<td>Albumine buffer</td>
<td>4.00</td>
<td>4.2</td>
<td>4.2</td>
<td>105</td>
</tr>
</tbody>
</table>

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Recovery experiments. Different amounts of the same urine subjected to the concentrating process. Dissolved in different amounts of buffer.

<table>
<thead>
<tr>
<th>Volume of urine dialysed ml</th>
<th>Re-dissolved volume of buffer ml</th>
<th>Assay value ng/ml</th>
<th>Expected ng/ml</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>3.3</td>
<td>3.4</td>
<td>97</td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>0.75</td>
<td>0.68</td>
<td>110</td>
</tr>
</tbody>
</table>

b. Table 2 shows recovery experiments when different amounts of the same urine are subjected to the concentrating process. Recovery was between 97–110%.

*Dilution experiments.* — The use of the radioimmunological method described above, revealed a factor in concentrated urine that with regard to binding to HGH antibodies, competes with $^{125}$I-HGH. Determinations on urine dilutions after concentrating the urine as described above, revealed a direct proportionality between the degree of dilution of the urine and the inhibition of binding of $^{125}$I-HGH to HGH antibodies over a wide range (dilutions up to 1:16). This was true in dilutions from a normal male (Fig. 2), and from two acromegalic patients (Fig. 3). The patient shown at the top in Fig. 3 had very

![Dilution of normal urine](image)

A urinary sample from a normal subject was subjected to the concentrating procedure described. A serial dilution was then performed and the IRHGH measured.
Dilution curves of urine from two acromegalic patients. At the top, the urine was diluted without any concentrating process, as the amount of IRHGH in the urine was very high. At the bottom, a urine from another acromegalic patient was subjected to the usual concentrating procedure and afterwards a serial dilution was performed.

high plasma IRHGH (704 ng/ml) and the dilution curve was performed without any previous concentrating procedure. However, there is still a proportionality between the degree of dilution of urine and the measured IRHGH.

Injection of exogenous HGH. – Fig. 4 shows plasma IRHGH and urinary IRHGH in a normal subject after a single im injection of HGH. It is demon-
Injection of HGH in normal person

Plasma IRHGH and urinary IRHGH after a single im injection of HGH. The solid line indicates plasma IRHGH and the hatched columns urinary IRHGH.

strated that the excretion of the urinary IRHGH increases proportionally to the plasma level. The urinary clearance calculated from this experiment is 0.006–0.01 ml/min.

*Gel filtration.* – As is seen from Fig. 5 IRHGH is regained in one peak after gel filtration on Sephadex G-100. The recovery ranges between 63–108%. It

400 ml of concentrated urine subjected to gel filtration (Sephadex G-100) and eluted with phosphate buffer 0.04 M pH 7.4 containing 0.9% NaCl and 1% human albumin. The columns indicate IRHGH. The elution volume of blue dextran, myoglobin and NaI is indicated.

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The excretion of IRHGH in urine in acromegaly, normal subjects and adult hypopituitarism. The legend on the right side (the acromegalic values) is $10 \times$ that on the left side (normal subjects and patients with hypopituitarism).

is demonstrated that IRHGH is recovered in an area corresponding to a molecular weight of approximately 20 000–30 000. $K_{\text{av}} = 0.40$. $K_{\text{av}}$ for $^{125}\text{I}$-HGH 0.43.

The excretion of IRHGH in urine in acromegaly, normal subjects and in adult hypopituitarism. – This is shown in Fig. 6. In normal subjects the mean is 41.3 ng/24 h (range 28–54 ng/24 h). No significant difference was demonstrated between males and females. Three patients with adult hypopituitarism were investigated. None of the patients had a plasma IRHGH response during insulin hypoglycaemia. In two of these patients, the urinary IRHGH was below the lower detection limit of the assay (0.39 ng/ml, i.e. 8–16 ng/24 h with a diuresis of 1000–2000 ml). The third had detectable amounts of IRHGH in the urine.

The excretion of IRHGH in the urine was augmented in all acromegalic patients studied as seen in Fig. 6 (Mean 1069 ng/24 h, range 89–3149 ng/24 h). The arrow points to an acromegalic who had a large pituitary tumour with suprasellar extension. A subtotal transfrontal hypophysectomy was undertaken and as is seen in Fig. 6, the urinary IRHGH decreased to about one sixth of its pre-operative concentration. The plasma IRHGH decreased from approximately 704 ng/ml to about 176 ng/ml. The other acromegalic patient
with an extremely high IRHGH concentration in the urine (2661 ng/24 h) had slight albuminuria. None of the other subjects in this study had albuminuria as evaluated by albustix.

**DISCUSSION**

By using the radio-immunological method described above, a factor in concentrated human urine was revealed that with regard to binding to HGH antibodies competed with ¹²⁵I-HGH. It has been demonstrated by Girard & Greenwood (1968) that increasing concentration of NaCl may inhibit the binding between ¹²⁵I-HGH and HGH antibodies and thus mimic IRHGH in a radio-immunoassay. This has been confirmed in the present study. However, it has also been demonstrated that the Na⁺ concentration following the concentrating process is below that shown to influence the radio-immunoassay to any significant extent. Urea in the concentration found in the urine after the concentrating process does not influence the assay. Thus it is not reasonable to assume that the inhibition of binding to HGH antibodies demonstrated is due to salts. Srivastava et al. (1971) showed that glucose might interfere in the double antibody radioimmunoassay for IRHGH. However, they pointed out that glucose did interfere with the precipitating reaction. Glucose in concentrations up to 5% did not affect the present assay. The reason for this discrepancy may be that in the present study the pre-precipitation technique was used; this means that glucose (and other substances that might be present in urine) is not introduced until the precipitating reaction has taken place.

Dilution curves show a proportionality between the degree of dilution and the demonstrated value. Girard & Greenwood (1968) demonstrated that by diluting their »unspecific urinary inhibition« more than 1:8, the percentage of bound radioactivity did not change. It is seen from the present experiments that the IRHGH is diluted proportionally to 1:16, then the concentration of IRHGH is at the lower limit of detection for the assay. The recovery studies are satisfactory although dialysis induces a small loss of IRHGH.

Girard & Greenwood (1968) concluded from recovery experiments that the IRHGH concentration in human urine was less than 100 ng/ml, but they were not able to determine the actual concentration. The present normal range (28–54 ng/24 h) agrees well with the suggested data of Girard & Greenwood (1968).

The molecular weight of pituitary HGH is approximately 21,700. Molecules of this magnitude are believed to allow free permeability over the glomerular membrane (Berggård 1970). Lowy et al. (1971) demonstrated that retardation at the glomerular membrane must play a minor role in determining GH ex-

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cretion in renal failure. They concluded that both insulin and HGH are avidly reabsorbed by the renal tubule. Collipp et al. (1966) have demonstrated the localization of GH in the proximal tubules of the rat kidney. However, it has been shown that nephrectomy in rats does not acutely change the half life of $^{125}$I-HGH (Rabkin et al. 1971). The half life did not increase until uraemia occurred in the nephrectomized rat. They concluded that although GH localizes in the kidney, this organ is unlikely to be directly responsible for much of the degradation of the hormone. On the other hand, Sönksen et al. (1971) studied the metabolic clearance rate of HGH in the dog and rhesus monkey. They stated that the renal clearance of HGH was approximately equal to the splanchic clearance and that they accounted for all the HGH metabolized. The urinary clearance of intact HGH averaged less than 0.25% of the renal extraction. Nevertheless, Sönksen et al. (1971) demonstrated that the urinary excretion of HGH in the dog appeared to be a linear function of HGH in the blood.

From the present study, it is evident that the urinary excretion of IRHGH is very low indeed. Less than 0.001% of the injected HGH is found in the urine as IRHGH. The urinary clearance calculated from the same experiment is between 0.006–0.01 ml/min. Nevertheless, it seems as if the IRHGH in urine reflects plasma levels in normal and pathological circumstances.

It is of interest that human placental lactogen (HPL) which in structure and molecular weight resembles HGH in great detail (Li 1972), is known to be excreted in human urine in relatively large quantities during late pregnancy (Grumbach et al. 1968). By calculating the urinary clearance for HPL from their figures, a value between 0.003–0.009 ml/min is obtained, i.e. in close agreement with the present results for HGH.

The present normal value for IRHGH in urine is below that estimated by previous investigators. Using a haemagglutination-inhibition method, Geller & Loh (1963) measured from 16.2–157 μg/24 h HGH in urine from normal subjects. However, Berson & Yalow (1964) demonstrated that this method, at least in plasma, was subject to unspecific inhibition. Sakuma et al. (1968) demonstrated a normal range for HGH in urine from 0.7–11.0 μg/24 h. However, they performed the radio-immunoassay on unconcentrated urine and did not consider the unspecific influence of salts on the assay. Recently Bala et al. (1971) demonstrated by a concentrating procedure similar to ours, a mean value of total IRHGH in urine in normal subjects of 79 ng/ml, i.e. in agreement with the present results.

In the present study, all acromegalic patients had an increased excretion of IRHGH in the urine. This observation is at variance with that of Glick (1968). By determining IRHGH on unconcentrated urine, no difference between normal adults and acromegalic patients was demonstrated. However, the values for normal subjects ranged between 0.2–0.9 ng/ml urine, i.e. about
20 times the levels in the present study. It is well known that some acromegalic patients may have a normal fasting plasma HGH (Burger & Catt 1969). One of the patients in the present study, who was grossly acromegalic, had on repeated occasions a normal fasting plasma HGH between 4–5 ng/ml. However, the excretion of IRHGH in the urine was clearly elevated (186 ng/24 h). Thus the estimation of IRHGH in urine may be of value in the diagnosis of acromegaly in certain situations. In three adult hypopituitary patients, a decreased excretion of IRHGH in urine was observed. In two patients the excretion was below the lower detection limit for the assay, but one had an excretion in the lower part of the normal range. It is well known that in many hypopituitary subjects the fasting plasma IRHGH is within the normal range so this might explain this finding. It remains to be seen whether the excretion of IRHGH in urine will be of any value in the diagnosis of hypopituitarism.

It is seen from Fig. 5 that the IRHGH of urine is retained in one fraction after gel filtration. IRHGH in urine is of high molecular weight, but it has not been clarified by the present work whether IRHGH in urine is due to intact pituitary HGH excreted in urine or to a metabolite of high molecular weight with retained immunological properties.

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REFERENCES


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