EFFECT OF HEAT ON THE IMMUNOLOGICAL PROPERTIES OF HUMAN, BOVINE AND OVINE GROWTH HORMONE

By

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ABSTRACT

Human, bovine and sheep (ovine) growth hormone (HGH, BGH and SGH) were heated in solution at temperatures between 60 and 100 °C. The electrophoretic mobility and immunological properties, such as precipitation reactions in agar gel and haemagglutination with antiserum to untreated hormone, were studied at different degrees of heating. It was found that heat progressively reduced the immunological properties of the growth hormone; however, human growth hormone was more resistant to heat treatment than the bovine and sheep growth hormone. HGH retained precipitation properties when heated at 100 °C up to 30 minutes, and reacted in the haemagglutination test when heated at 100 °C for less than 60 minutes. BGH and SGH clotted at 100 °C. The precipitation reaction with antiserum to BGH disappeared when BGH or SGH was heated at 70 °C for more than 10 minutes. Only a weak haemagglutination reaction was retained when BGH or SGH was heated at 80 °C for 15 minutes.

With the increasing use of immunological methods for the measurement of growth hormone concentration in biological fluids, it seems of great importance to learn more about the immunological properties of this hormone. In previous studies (Laron et al. 1964) it was shown that enzyme digestion progressively reduced the immunological properties of human as well as of bovine growth


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hormone. A weak haemagglutination reaction was found even after 60 to 75 % digestion, demonstrating a high degree of immunological stability of growth hormone. The present investigation was designed to find out the effect of heat on the immunological properties of growth hormone.

**MATERIALS AND METHODS**

**Growth hormone preparations**

Human growth hormone (HGH) was prepared from pituitary glands obtained at autopsy and preserved in acetone according to the method of Raben (1959); one batch A-4 was used. Bovine growth hormone (BGH) and sheep (ovine) growth hormone (SGH) were obtained from the Endocrinology Study Section of the U.S. National Institute of Health and prepared by Dr. A. E. Wilhelmi; batches NIH-GH-B-2 and NIH-GH-S-4 were used.

**Immunological techniques**

The preparation of antisera and the procedure of agar gel double diffusion, electrophoresis and immunoelectrophoresis were performed as previously described (Laron & Assa 1962). Haemagglutination was performed using human red blood cells (RBC) type 0 and normal rabbit serum (NRS). The red blood cells were treated with tannic acid according to Boyden (1951) and Stavitsky (1954). The treatment with tannic acid of the RBC was performed in a 2.5 % suspension in pH 7.2 phosphate buffered saline at 37 °C for 10 minutes. The sensitization of the tannic acid treated RBC with HGH was performed in the same buffer adding HGH (1 mg in 6 ml) to the tanned RBC suspension volume per volume. The RBC were incubated at 37 °C for 20 minutes. After the incubation the RBC were washed several times in 1/100 NRS. The RBC were freshly prepared before each experiment. Bovine and sheep (ovine) growth hormones are immunologically identical (Hayashida & Li 1959) and thus antiserum to BGH was used for both hormones.

**Heating**

In a typical experiment, a series of test tubes containing 2 mg hormone dissolved in 0.2 ml water with the addition of 1 N NaOH were heated in a water bath at the desired temperature. At various periods the electrophoretic and immunological properties of the heated hormone were tested. Non-heated hormone served as control.

**RESULTS**

Human Growth Hormone (HGH)

Human growth hormone was heated at 100 °C and tested at 10 minute intervals up to one hour. Cloudiness was first observed after 40 minutes and increased with prolonged heating.

**Agar gel electrophoresis**

The electrophoretic mobility of heated HGH is illustrated in Fig. 1. It is seen that whereas the mobility of non-heated HGH corresponds to an area between
Electrophoretic mobility in agar gel of unheated and heated (100° C) human growth hormone (HGH) as compared with human serum. Photograph taken after staining with amino-black. The arrows point to the origin of the electrophoresis.

Interaction between human growth hormone (HGH) unheated and heated at 100° C and HGH antiserum, as determined by the Ouchterlony agar gel double diffusion technique. Photograph taken after staining with amido-black.

beta and alpha globulin, heated HGH moved towards the anode corresponding to the area of albumin. The mobility of HGH increased with the time of heating but the intensity of its staining decreased.

Agar gel double diffusion (Ouchterlony) and agar gel immunoelectrophoresis

Tested against antiserum to untreated HGH, HGH heated at 100° C for 30 minutes gave one precipitation line, which joined with the precipitation line
Interaction between human growth hormone (HGH) unheated and heated at 100° C and HGH antiserum as determined by agar gel immunoelectrophoresis. Photograph taken after staining with amido-black. The arrows point to the origin of the electrophoresis.

produced by the untreated hormone (Fig. 2). Heating for longer than 30 minutes led to the disappearance of the precipitation line. The same was found in agar gel immunoelectrophoresis (Fig. 3). Whereas the precipitation line of untreated hormone appeared one day after its application, the precipitation line of the HGH heated for 30 minutes only became visible after three days.

Haemagglutination

The results obtained by testing heated HGH with antiserum to untreated HGH are illustrated in Table 1. It is evident that with prolonged heating there

Table 1.

Haemagglutination reaction between heated human growth hormone (HGH) and antiserum to HGH.

<table>
<thead>
<tr>
<th>Antigen: HGH 1/100 Heated at 100° C</th>
<th>Dilution of antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/120</td>
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<tr>
<td>0 minutes</td>
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<tr>
<td>10 &quot;</td>
<td>+++</td>
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<tr>
<td>20 &quot;</td>
<td>+++++</td>
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<td>30 &quot;</td>
<td>+++</td>
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<tr>
<td>40 &quot;</td>
<td>++</td>
</tr>
<tr>
<td>50 &quot;</td>
<td>+</td>
</tr>
<tr>
<td>60 &quot;</td>
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</tr>
</tbody>
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Electrophoretic mobility in agar gel of unheated and heated bovine (BGH) and sheep growth hormone (SGH). Photograph taken after staining with amido-black.

is a progressive reduction in the haemagglutination capacity of HGH up to 60 minutes heating at 100° C when this property completely disappears.

Bovine and Sheep Growth Hormone (BGH, SGH)
Bovine growth hormone heated at 100° C coagulated after several minutes and became unsuitable for testing. Tests were therefore performed, heating the hormone at 60° C, 70° C and 80° C for various periods of time. Heating at 80° C for 15 to 30 minutes caused some turbidity of the dissolved hormone solution. After 60 minutes at 80° C a gel clot was obtained.

Agar gel electrophoresis
Both BGH and SGH heated at 60°, 70° or 80° C lost their electrophoretic mobility and as judged by the staining intensity remained at the origin of application (Fig. 4).

Agar gel double diffusion (Ouchterlony) and agar gel immunoelectrophoresis
Untreated BGH or SGH tested against antisera to BGH caused the appearance of two to three precipitation lines; heating at 60° C for 10 to 30 minutes, and at 70° C for 10 minutes resulted in the appearance of one precipitation line only. Raising the temperature and prolonging the time of heating delayed the appearance of the precipitation line. The precipitation line no longer appeared after heating at 70° C for more than 15 minutes, or at a higher temperature.
Table 2.

Haemagglutination reaction between heated sheep growth hormone (SGH) and antiserum to bovine growth hormone (BGH).

<table>
<thead>
<tr>
<th>Antigen: SGH 1/100 Heating</th>
<th>Dilution of antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temp.</strong></td>
<td><strong>Time in minutes</strong></td>
</tr>
<tr>
<td>0</td>
<td>0'</td>
</tr>
<tr>
<td>60°C</td>
<td>10'</td>
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<tr>
<td>60°C</td>
<td>30'</td>
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<td>70°C</td>
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<tr>
<td>70°C</td>
<td>20'</td>
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<tr>
<td>75°C</td>
<td>10'</td>
</tr>
<tr>
<td>80°C</td>
<td>15'</td>
</tr>
<tr>
<td>80°C</td>
<td>40'</td>
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</tbody>
</table>

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**Haemagglutination**

As illustrated in Table 2, both increase in the temperature and period of heating progressively reduced the haemagglutination property of sheep growth hormone.

**DISCUSSION**

Few studies on the effect of heat on growth hormone have been performed. Li *et al.* (1945) reported that bovine growth hormone immersed in boiling water for 10 minutes lost its growth promoting activity. Recently Li (1962) found that unlike bovine hormone there was no decrease in the biological activity of human growth hormone after being kept at 100°C for 15 minutes. To the best of our knowledge no investigation on the immunological properties of growth hormone after heating have been reported previously.

In the present study it became evident that heat which is known to cause denaturation of the protein molecule (Putnam 1953) progressively reduced the immunological properties of human, bovine and sheep growth hormone as measured by gel diffusion and haemagglutination tests and also changed the electrophoretic mobility of these hormones. Bovine and sheep growth hormones are more sensitive to heat treatment than the human hormone, which retains the property of haemagglutinating sensitised red cells in the presence of antisera against untreated HGH, heated at 100°C for 50 minutes. Bovine and sheep growth hormone clotted at 100°C and had to be tested at 80°C or less. Heating at 60°C for as little as 10 minutes markedly reduced their haemagglutination properties.

The chemical structure of the growth hormones is not yet known and hence no comment can be made on the difference in immunological stability to heat between the human and bovine or sheep growth hormone.

**ACKNOWLEDGEMENT**

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**REFERENCES**


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