According to Gurin, Bachman & Wilson (1940) the carbohydrate in chorionic gonadotrophic hormone is believed to be composed of hexose and hexosamine in the proportion 2:1, the total sugar amounting to 17.9 per cent. After hydrolysis of the preparation with 0.1 N sulphuric acid at 100° for 6 hrs Gurin et al. found the hexose to consist of galactose, which was identified as methylphenylhydrazone.

The accuracy of the paper partition chromatography technique, as applied by Partridge (1946) to the identification of sugars, seemed very suitable for the qualitative analysis of the carbohydrate components in human chorionic gonadotrophic hormone.

Attempts to isolate sugars by the paper chromatographic technique from acid hydrolysates of commercial human chorionic gonadotrophic hormone (HCG) preparations were unsuccessful even after making extensive use of ion exchange resins for the removal of disturbing inorganic matter.

With the technique described by Gurin, Bachman & Wilson

*) The costs of these investigations have been met by a grant from Sigrid Jusélius Stiftelse.
in 1939, HCG from 12 liter of pregnancy urine was then preci-

tipated with benzoic acid and acetone and repeatedly purified
by extraction with 50 per cent ethanol and precipitation with
absolute ethanol at a different pH. The last precipitate, about
200 mg. of a greyish-white substance, was then hydrolized in
1 N sulphuric acid for 3 hrs at 100° C. After removal of the
inorganic matter with desalting resins (Light & Co) the residue
was evaporated to a small volume and drops (0.02 ml.) of
this solution applied on the filter paper strip (Whatman No 1).
The chromatogram was run in a cabinet with butanol-acetic
acid (butanol 4 : acetic acid 1 : water 5) as the solvent (de-
scending technique). Developing of the chromatogram with
benzidine (Horrocks, 1949) after 24 hrs definitely showed
galactose (Rf 0.16) to be a regular constituent of the hydro-
lysate. Others sugars could not be observed on the chromato-
grams.

Fifty mg. of a crystalline preparation of HCG (Cloesson
et al., 1948) which was stated to possess an activity of 6000—
8000 I. U. per mg. was then analyzed.*) As hydrolysis with
1 N sulphuric acid at 100° C for 3 hrs yielded only galactose,
this preparation was hydrolyzed with 2 N sulphuric acid at
100° C for 3 hrs. The hydrolysate was neutralized with
Ba (OH)₂ to pH 6.5. After centrifuging, the supernatant was
evaporated to about 2 ml. and drops (0.02 ml.) of this solution
applied on filter paper strips (Whatman No 1). The chromato-
grams was run in cabinets with butanol-acetic acid or pyridine-
amyl-alcohol (Edman, 1945) as solvents (descending tech-
nique). In addition to numerous spots with reference sugars
and glucosamine, drops containing unhydrolyzed HCG in water
solution were applied on filter paper strips.

The chromatograms were observed in ultraviolet light**) be-
fore developing. Corresponding to the applied drop of un-

*) The hormone was kindly put at our disposal by AB Leo, Häl-
singborg by courtesy of Professor A. Westman.

**) The observations in ultra-violet light was made possible by
courtesy of the Director of The Centrallaboratorium in Helsinki, Dr.
B. Nybergh, and his assistants Dr. J. Sundman and J. Saarnio.
hydrolyzed hormone in water solution there was a bright fluorescence distinctly limited to this starting point on the paper. The solvents used did not produce any source of fluorescence. After hydrolysis the fluorescence around the starting point had considerably decreased. There was now slight fluorescence all the way down to Rf 0.11 (in butanol-acetic acid) where a bright and maximum of fluorescence could consistently be seen. This fluorescence at Rf 0.11 (in butanol-acetic acid) corresponded exactly to the fluorescence at the same Rf 0.11 which could only be due to glucosamine applied in reference spots on the same starting line. The correctness of the observation that the fluorescence at Rf 0.11 was due to glucosamine from the hydrolysate was then confirmed by developing the papers with ninhydrin (0.1 per cent in butanol) and by the test described by Elson & Morgan (1933). The violet colour with ninhydrin and the greyish cherry red colour with Elson's & Morgan's test both indicated glucosamine at Rf 0.11 from the hydrolysate and in the reference spots.

There was some fluorescence from the hydrolysate below Rf 0.11 with faint but regular maxima at Rf 0.20, 0.45 and 0.53 in butanol-acetic acid. With ninhydrin no colour could be observed at these points.

In pyridine-amylalcohol, glucosamine from the hydrolysate could be identified at Rf 0.16. In this solvent there was irregular bright fluorescence down to Rf 0.16 and two or three maxima at Rf 0.30—0.40.

The nature of this fluorescence is being further investigated.

The strips were then developed for other sugars. With absolute regularity galactose and mannose could be identified from the hydrolysate independently of the solvent or developer. Benzidine (Horrocks, 1949), aniline hydrogen phthalate (Partridge, 1949) and silver nitrate (Partridge, 1946) all revealed the presence of galactose, mannose and glucosamine in typical Rf-positions as shown in Table 1 and in Fig. 1. The spots produced by galactose were always very distinct whereas
Table 1.

Rf-values of galactose and mannose from a hydrolysate of human chorionic gonadotrophic hormone and of some reference sugars.

<table>
<thead>
<tr>
<th>Hydrolysate:</th>
<th>Butanol-acetic-acid-water 4:1:5</th>
<th>Pyridin-amylalcohol-water 33:35:30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>0.14—0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.20</td>
<td>0.33</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>0.11</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference sugars:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.20</td>
<td>0.33</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Glucosamine</td>
<td>0.11</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The spots due to mannose were considerably fainter. When taking only about 0.005 ml. of the hydrolysate instead of the usual 0.02 ml. for the drop to be applied on the paper, the spots produced by galactose was still very distinct whereas no spot at all in the position of mannose could be observed. The presence of smaller quantities of mannose than galactose in the chorionic gonadotrophic molecule rather than incomplete hydrolysis may well be the cause of these spots produced by mannose from the hydrolysate.

The remainder of the hydrolysate was then further hydrolyzed (for analysis of amino acids) with 5 N sulphuric acid for 29 hrs at 100° C and the hydrolysate again neutralized with Ba (OH)₂ to pH 6.5, centrifuged and evaporated to a small volume. Chromatograms now run in the same solvents and developed with aniline hydrogen phthalate and benzidine still showed the presence of galactose, mannose and glucosamine, the spot due to mannose still being the faintest.

When this investigation was in progress Werner & Odin (1949) published similar results about the sugars in chorionic gonadotrophic hormone. They believed that galactose, mannose and glucosamine, in equimolar amounts, made up the carbohydrate part of the hormone.
Fig. 1.


SUMMARY

By means of paper partition chromatography after acid hydrolysis of human chorionic gonadotrophic hormone galactose, mannose and glucosamine are found to be constituents of the hormone molecule. The investigation indicates that considerably more galactose than mannose is present in chorionic gonadotrophic hormone.
REFERENCES


