STUDIES WITH THYROTROPHIN-\textsuperscript{131}I IN SERUM
OF NORMAL AND HYPOTHYROID RATS

By

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ABSTRACT

Purified bovine thyrotrophin (TSH-NIH) was labelled with \textsuperscript{131}I. Its radiolysis was studied by paper chromatography. The results obtained indicated that the preparation should be used within the first few days of the labelling procedure.

The labelled hormone was injected intravenously into normal and hypothyroid rats. The serum disappearance rate of TSH was lower in the hypometabolic group and the curve showed three components. The half life of the second component was about 14 minutes for normal and 21 minutes for hypothyroid rats.

Column chromatography of sera developed with Dowex 1 $\times$ 8 showed three fractions. The significance of these is discussed.

Few studies have been done on the circulation of thyrotrophin (TSH) in the serum. Mancini \textit{et al}. (1961) showed that fluorescent TSH moved with bands corresponding to the $\alpha$- and $\beta$-globulins. Bakke (1963) studied the serum half life of non-labelled TSH in rats in different metabolic states. In this study TSH was assayed by biological methods. All these studies, however, were done with non-physiological amounts of TSH.

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Recent advances in the techniques of purification and labelling of protein hormones have resulted in more purified TSH preparations labelled with iodine in high specific activities and with little loss of biological properties (Utiger et al. 1963; Lawrence-Heideman et al. 1965) thus allowing of the administration of physiological doses of TSH.

This paper presents studies done on the serum of normal and hypothyroid rats after the injection of purified labelled thyrotophin.

MATERIALS AND METHODS

Female Wistar rats weighing about $180 \pm 30$ g were used. These studies were performed on 34 normal and 41 hypothyroid rats. These latter animals were made hypothyroid by radioiodothyroidectomy with 1 mc $^{131}$I, given i. p., and used four months later. Thyroidectomy was checked in each rat at sacrifice.

The bovine TSH used was generously provided by Dr. A. E. Wilhelmi, N. I. H., B1, 4 ILU/mg and this preparation showed a single band by immunoelectrophoresis and by disc gel acrylamide electrophoresis (Pisarev et al. 1967). The TSH was labelled according to the method of Greenwood et al. (1963) in Division of Labelled Molecules (Atomic Energy Commission, Argentina) and used within two days of labelling. Its specific activity ranged between 30–80 mc/mg (Altschuler et al., unpubl. results).

In order to study the radiolysis of the labelled compound, ascending chromatographic runs were done in methanol:water system (70:30 v/v) for 4 h on different days after TSH-$^{131}$I was obtained. The iodide present in each paper strip was measured by scanning. All the labelled preparations used in these studies contained less than 5 % of iodide.

The rats were injected intravenously in the left femoral vein with 0.5 $\mu$c of TSH-$^{131}$I in 0.5 ml of saline. This amount corresponds to 0.05 mU of TSH. The animals were sacrificed at different times between 2 and 240 min after the TSH injection, by cardiac puncture under light ether anaesthesia.

Serum radioactivity was measured in a well-type scintillation counter. Column chromatographic studies were carried out using the resin Dowex $1 \times 8$ (20–50 mesh) (Dow Chemical Corp., U. S. A.) equilibrated with acetate buffer pH 5.5, ionic strength 0.1. The elution of radioactive fractions was first carried out with this buffer and then with glacial acetic acid. By this procedure iodide remains adsorbed to the resin (Altschuler et al. 1964).

RESULTS

Table 1 shows the results obtained by serial paper chromatographic studies of TSH-$^{131}$I on different days after the labelling procedure. On the 4th day, about 9.5 % of iodide is present and the preparation can thus no longer be used.

The column chromatographic study demonstrates the presence of three fractions: I, eluted with acetate buffer which represents the main peak of radioactivity and which decreases with time; II, eluted with glacial acetic acid, the values of which remained at all times at about 1–2 % of serum radioactivity,
Table 1.
Radiolysis of TSH-\(^{131}\)I.

<table>
<thead>
<tr>
<th>Days after labelling procedure</th>
<th>% of labelled hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>96.5</td>
</tr>
<tr>
<td>4</td>
<td>90.5</td>
</tr>
<tr>
<td>6</td>
<td>85.5</td>
</tr>
<tr>
<td>8</td>
<td>85.0</td>
</tr>
<tr>
<td>10</td>
<td>82.0</td>
</tr>
<tr>
<td>15</td>
<td>81.0</td>
</tr>
</tbody>
</table>

and III, the iodide, which remained adsorbed to the resin. Recovery values were always around 95–102 %\(_{0}\). The sum of fractions I and II was expressed as a percentage of the administered dose per ml of serum. The values thus obtained were plotted against time and can be seen in Fig. 1, which shows the curves of TSH-\(^{131}\)I disappearance from the serum of normal and hypothyroid rats. The rate of decrease of radioactivity was higher in the normal than in the hypometabolic group. There seemed to be three components in the curve. The first one, corresponded to the initial dilution of the injected hor-
mone. The half life of the second component calculated for both groups, was around 14 min in the normal and 21 min in the hypothyroid rats.

**DISCUSSION**

Previous studies carried out in our laboratory (Pisarev et al. 1967) showed the high degree of purity of the TSH preparation used in these studies. The amount of TSH administered with the labelled hormone was within the physiological range of circulating TSH in the rat (Panda & Turner 1967).

The studies done on the radiolysis of the labelled compound indicate that this preparation must be used within the first few days of the procedure.

The decrease of radioactivity was very slow in the serum of the hypothyroid rats. The same results were observed by us when studying the TSH-131I distribution in different organs (Altschuler et al., unpublished results).

The curve of disappearance shown in Fig. 1 can be divided into three components. The first part has a rapid fall in radioactive values which may be due to the initial dilution in plasma volume. The second part of the curve is related to serum clearance of TSH. At these times high values were observed in kidney radioactivity (Altschuler et al., unpublished results) and similar results were obtained by Bakke (1963). The third component is characterized by a slow decrease in radioactivity and probably represents true metabolism of the hormone.

The half life values for the second component are higher in the hypothyroid group. Bakke (1963) calculated the serum half life of TSH and also found the same difference. Werner (1963) stated that non-physiological amounts of TSH injected by Bakke (1963) could exceed the renal threshold thus giving half life values which only reflect the effect of excessive doses. However, our results agree very well with those of Bakke (1963) and the amount of labelled hormone injected was within the physiological range for the rat. D'Angelo (1963) pointed out that the curve of disappearance may have more than one component. These conclusions agree with our results.

Fraction I, obtained by column chromatography, represents the main part of the labelled hormone and shows a decrease with time. The significance of the second fraction remains unexplained, and its values remained, at all the times studied, at the same level. According to the hypothesis of Antoniades et al. (1965) one would suspect that this fraction corresponds to the »free« hormone. The constancy of its values supports this assumption. However, this fraction may represent »damaged« TSH, as reported by Yalow & Berson (1966) for other labelled hormones, or a iodinated peptide product of TSH-131I degradation. Final conclusions about this important subject must await further studies.

505
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REFERENCES


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506