Activity Ratio of Adrenaline to Noradrenaline in Various Colour Reactions

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In estimating adrenaline content of the adrenal medullary tissue or of the adrenal venous blood, various colour reactions have been applied, adrenaline being used as the reference standard. However, it is generally accepted that noradrenaline is contained besides adrenaline in the adrenal medulla or in the adrenal venous blood. In this connection it is of great interest to clarify the colorimetric activity of noradrenaline in various colour reactions.

EXPERIMENTAL

Methods and Results

The permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and the arsenomolybdic acid methods found their application.

Synthetic L-adrenaline and DL-noradrenaline (Sankyo) were used as the samples. These were dissolved with N/10 HCl in 1:1,000, and further diluted if necessary. Using the respective methods, a known amount of adrenaline or noradrenaline was coloured and the colour density of noradrenaline was compared with that of adrenaline. No difference was causable by employing L-noradrenaline (Sterling Winthrop) instead of DL-noradrenaline.

Experimental results are tabulated.

The permanganate method:

The procedure used in this test was almost the same as that described by Zanfrognini. In the present studies, the colour reaction was performed at constant temperature (20°C) and pH (6.0) to avoid effects causable by changes of temperature and pH. The colour tint of noradrenaline after oxidation with the permanganate reagent was approximately similar to that of adrenaline.

The colorimetric activity ratios of adrenaline to noradrenaline ob-
TABLE I

Colorimetric Activity Ratios of Adrenaline to Noradrenaline in Various Chemical Methods

<table>
<thead>
<tr>
<th>No. of exp.</th>
<th>Permanganate method</th>
<th>Corrosive sublimate method</th>
<th>Iodine method</th>
<th>Phosphotungstic acid method</th>
<th>Arsenomolybdic acid method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.16:1</td>
<td>1.30:1</td>
<td>2.3:1</td>
<td>2.20:1</td>
<td>7.2:1</td>
</tr>
<tr>
<td>2</td>
<td>1.16:1</td>
<td>1.32:1</td>
<td>2.3:1</td>
<td>2.20:1</td>
<td>7.8:1</td>
</tr>
<tr>
<td>3</td>
<td>1.15:1</td>
<td>1.29:1</td>
<td>2.3:1</td>
<td>2.18:1</td>
<td>7.8:1</td>
</tr>
<tr>
<td>4</td>
<td>1.17:1</td>
<td>1.32:1</td>
<td>2.5:1</td>
<td>2.11:1</td>
<td>7.9:1</td>
</tr>
<tr>
<td>5</td>
<td>1.17:1</td>
<td>1.30:1</td>
<td>2.3:1</td>
<td>2.15:1</td>
<td>7.1:1</td>
</tr>
<tr>
<td>6</td>
<td>1.17:1</td>
<td>1.31:1</td>
<td>2.5:1</td>
<td>2.18:1</td>
<td>7.6:1</td>
</tr>
<tr>
<td>7</td>
<td>1.16:1</td>
<td>1.32:1</td>
<td>2.3:1</td>
<td>2.15:1</td>
<td>8.0:1</td>
</tr>
<tr>
<td>8</td>
<td>1.16:1</td>
<td>1.28:1</td>
<td>2.3:1</td>
<td>2.20:1</td>
<td>7.6:1</td>
</tr>
<tr>
<td>9</td>
<td>1.15:1</td>
<td>1.32:1</td>
<td>2.3:1</td>
<td>2.19:1</td>
<td>7.6:1</td>
</tr>
<tr>
<td>10</td>
<td>1.14:1</td>
<td>1.29:1</td>
<td>2.5:1</td>
<td>2.17:1</td>
<td>7.3:1</td>
</tr>
<tr>
<td>Mean</td>
<td><strong>1.16:1</strong></td>
<td><strong>1.31:1</strong></td>
<td><strong>2.4:1</strong></td>
<td><strong>2.17:1</strong></td>
<td><strong>7.6:1</strong></td>
</tr>
</tbody>
</table>

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The corrosive sublimate method:

The method of Suto and Inouye (modified by Kojima) was applied. In this reaction, the colour tint of noradrenaline after oxidation was mostly similar to that of adrenaline.

The activity ratios of adrenaline to noradrenaline obtained in 10 experiments were 1.14:1–1.17:1, the average being 1.16:1.

The iodine method:

This colour reaction was carried out according to the description of Euler. In this iodine method, however, it was noticed that the colour tint differed remarkably between adrenaline and noradrenaline; that is, brownish red in adrenaline and purplish red in noradrenaline. Furthermore, the colour development of noradrenaline varied correspondingly to the changes of the reaction temperature.

Therefore the following procedure was contrived: 1) Colorimetric comparisons were made after adding to noradrenaline some amount of adrenaline enough to make the colour tint similar to that of adrenaline. 2) The colour reaction was carried out at the constant temperature of 20°C in order to remove the effects causable by the changes of the reaction temperature.

The activity ratios of adrenaline to noradrenaline obtained in 10 experiments were 2.3:1–2.5:1, and the mean value was 2.4:1. The somewhat greater variability of these values seems to be due to the difference of colour tint between adrenaline and noradrenaline and to the procedure
contrived to reduce it.

The phosphotungstic acid method:

The method of Folin, Cannon and Denis was used. In my experiments, however, the colour densities of adrenaline and noradrenaline were compared colorimetrically directly without using uric acid as the standard.

The colour of noradrenaline obtained in this reaction had a little more dark-brownish tone than that of adrenaline. Nevertheless, the colorimetric comparison was practically not so difficult.

The activity ratios of adrenaline to noradrenaline were found in 10 experiments to be 2.11:1–2.20:1, and the average was 2.17:1.

The arsenomolybdic acid method:

This colour reaction was performed, using the method of Bloor and Bullen. The colour tint of adrenaline and noradrenaline obtained in this reaction was approximately similar. The colorimetric activity ratios of adrenaline to noradrenaline were estimated to be 7.1:1–8.0:1, the average being 7.6:1.

The considerable variability of these values may be due to the complexity of procedure itself.

**DISCUSSION**

In the present investigation, the colorimetric activity ratio of adrenaline to noradrenaline was estimated by the following 5 chemical methods—the permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and the arsenomolybdic acid methods.

From my experimental results it is assumed that the disharmony of adrenaline estimates yielded by various methods as was found by the previous investigators (Kojima et al., Hatano and Arai) may be due to the difference of the activity ratio of adrenaline to noradrenaline in each method. This was already suggested by Suzuki, Nakamura and Ninagawa in their study on the biological activity ratio of adrenaline to noradrenaline. We should obtain lower adrenaline estimates in the chemical methods in which the activity ratio of adrenaline to noradrenaline is higher, if only noradrenaline is contained besides adrenaline in the adrenal medulla. However, experimental results reported by Kojima et al., Hatano and Arai are not fully explainable from my present data. It must be taken into account that the activity ratio of adrenaline to noradrenaline in various chemical methods may be influenced by the variations of the reaction temperature and of pH. Therefore this problem is to be re-investigated, taking the experimental conditions into consideration.

Some authors reported the activity ratio of adrenaline to noradrenaline in Shaw's arsenomolybdic acid method. In the experiments of Shaw, it was found to be 16:1. By the hand
of West\textsuperscript{12}, it was estimated to be 13.5:1. And it was estimated by Verly\textsuperscript{13} to be about 5:1. Gaddum, Peart and Vogt\textsuperscript{14}, on the other hand, obtained just the same value as Shaw.

**Summary**

Activity ratios of adrenaline to noradrenaline in various chemical methods, such as the permanganate, the corrosive sublimate, the iodine, the phosphotungstic acid and arsenomolybdic acid methods were estimated.

The mean value (adrenaline: noradrenaline) obtained was 1.16:1 in the permanganate method, 1.31:1 in the corrosive sublimate method, 2.4:1 in the iodine method, 2.17:1 in the phosphotungstic acid method and 7.6:1 in the arsenomolybdic acid method.

I hereby express my sincere gratitude to Prof. T. Suzuki for his interest in this work.

**References**

2) Suto, K. and Inouye, K., Ikai Jiho, (Japanese) 1921, 414.