Determination of Urine Dopamine

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The Duliere and Raper conversion of dopamine to 5,6-hydroxyindole is applied to urinary dopamine, which is concentrated and recovered by adsorption (pH 8.5-9.0) on and elution with dilute acetic acid from alumina. The spectrofluorometric readings are made at pH 5.3, with activation and fluorescence at 320 and 375 mjz, respectively. Proportionality between high and low internal standards is achieved when the 5,6-hydroxyindole reaction proceeds for 20 hours at room temperature.

Urinary dopamine was determined in normal individuals asleep and awake and in those with a number of pathologic conditions. The mean values were decreased in groups with Parkinsonism, diabetes, and cirrhosis of the liver. The study included 2 patients with pheochromocytoma.

Carlsson and Waldeck (1) have described a fluorometric procedure for the determination of dopamine. This is similar to the trihydroxyindole method for determining adrenaline and noradrenaline, but eliminates interference by the latter substances by the utilization of differences in fluorescence characteristics at a pH of about 5.3. Drujan et al. (2) modified this technic for the determination of urinary dopamine by taking fluorescent readings at a more acid pH.

The purpose of this article is to report on comparison of these two procedures as applied to determination of urinary dopamine, and to describe in detail a procedure of our own which is based on this experience, and in which certain errors or erratic results that may occur in
the older procedures are eliminated. A previously described procedure (3) for the isolation of adrenaline from urine has also been utilized for dopamine, yielding a state of purity amenable to the fluorescent determination.

Our method for determining dopamine, based on observations originally made by Duliere and Raper (4), consists of (Step 1) oxidation of dopamine by iodine (1) to a red indole derivative, which in the absence of oxygen in sodium hydroxide (Step 2) undergoes molecular rearrangement with formation of 5,6-dihydroxyindole. Step 3 involves adjustment to pH 5.3. At this pH or the more acid pH used by Drujan et al., the activity and fluorescent wavelengths of 5,6-dihydroxyindole are much lower than those of adrenolutine or noradrenolutine, so that the latter substances introduce no serious error in the determination. Furthermore, the yield of 5,6-dihydroxyindole or a related compound with the same optical properties increases with time. The activation and fluorescence peaks recommended by Carlsson and Waldeck (1) were 345 and 410 mμ (uncorrected). We found that when the spectrophotofluorometer was recalibrated against a mercury line spectrum, the maxima at pH 5.3 were 320 and 375 mμ, respectively. Drujan et al. (2) reading at pH 1.0, found that 330 and 375 were the maxima. These were reported as uncorrected.

Our procedure employs a blank in which the urine eluate is oxidized with iodine as usual, but sulfite is omitted during the rearrangement in NaOH, resulting in the destruction of the fluorescent derivative of dopamine. This procedure is included in the method of Carlsson and Waldeck (1), but is not employed by Drujan et al. (2), who resort to a reagent blank. The use of the reagent blank implies that dopamine is the only substance in the eluate forming a fluorescent derivative at the wavelengths read. The eluate blank implies that dopamine is the only substance the fluorescent derivative of which, at the wavelengths read, is destroyed in the absence of sulfite. It therefore adds specificity to the test, at the same time correcting for nonspecific fluorescent species in the eluate. These are readily demonstrated by comparing a reagent blank with an eluate blank, with both subjected to alkali without sulfite.

**Drujan Procedure (pH 1.1)**

These authors state that by acidifying the samples after the iodine reaction with 5N acid and allowing them to stand 45 min., interfering fluorescent substances decay and stable readings are obtained. In com-
paring the original procedure of Carlsson and Waldeck with that of Drujan et al., we find that the fluorescence after 45 min. continues to increase during the next 20 hours, whether the pH is 1.1 or 5.3, and the ratio of unknown to blank is virtually the same for both procedures. This illustrates that the lower pH does not have a lower blank reading in relation to the dopamine reading. The lower absorbance at the acid pH, therefore, reflects a decrease in accuracy. For 8 urine samples tested simultaneously by both procedures, the ratios of blanks (pH 5.3/pH 1.1) were 2.7 ± 0.2 and 2.1 ± 0.2 for 45 min. and 20 hours; the ratios for unknowns for the same periods were 2.3 ± 0.3 and 2.6 ± 0.3. The blank (7 urines) read at 20 hours, as compared with 45 min., for pH 5.3 was 109 ± 5 per cent. The difference was less than twice the instrument error. For pH 1.1 this figure was 150 ± 10 per cent for a comparable period. The difference is more than twice the instrument error. Here again the use of the more acid pH reflects no advantage.

Present Procedure

The reduction of the reaction time in iodine from 5 to 3 min. (Carlsson et al. used 5 min., Drujan et al. 3 min.) is an improvement as there is some destruction of chromophore after 3 min. A comparison of this timing for 7 urine samples indicated that the 3-min. period produced roughly 10 per cent higher values for the unknowns, when the readings were made at pH 5.3. In comparing standards added to 7 urines, the value for 5 min. was 86 ± 3 per cent the value for 3 min. This difference was the same whether the readings were made 15 min. after destruction of iodine or 20 hours later. The 3-min. period in iodine was chosen as the standard procedure.

We confirm the findings of Carlsson and Waldeck with respect to the degree to which noradrenaline interferes with the determination of dopamine. Comparison was made of 2 µg. dopamine with 2 µg. noradrenaline, both having been added to aliquots of the same urine. For 7 urines, noradrenaline gave 3 per cent the fluorescence of dopamine.

The procedure adopted for separation and purification of dopamine is essentially the same as that we reported (3) for adrenaline and consists of adsorption on alumina at pH 8.5-9.0 and elution with acetic acid after washing with sodium acetate. A comparison of 2-µg. dopamine standards added before and after elution of urine samples gave a mean recovery of 98 ± 1.7 per cent.

The ratios of readings for 2 and 4 µg. dopamine added to urine
aliquots and processed by the standard elution and reaction proce-
dures were 0.61 ± .023 when readings were made at 15 min., and 0.51 ± .016 when readings were made at 20 hours (11 different urines). This indicated 20 hours as the correct time for reading in the standard procedure. The blank reading increased 17 ± 3 per cent (14 samples) when read 20 hours after the destruction of iodine, as compared with an initial reading at 5 min. Half of this increase occurred the first 45 min. Since the dopamine reading doubled between 45 min. and 20 hours, an advantage in accuracy is again attained by reading at 20 hours.

Materials and Method
Reagents
- **Iodine solution, 0.2N.** Dissolve 0.254 gm. iodine and 5 gm. KI in H₂O and dilute to 100 ml.
- **Sodium hydroxide, 5 N.**
- **Sodium hydroxide, 4.5 N.**
- **Acetic acid, 5 N.**
- **Sodium acetate, 0.2 M.**
- **Alkaline sulfite solution.** Dissolve 2.52 gm. anhydrous Na₂SO₃ in 10 ml. H₂O and dilute with 5N NaOH to 100 ml.
- **Sodium sulfite.** Dissolve 2.52 gm. anhydrous Na₂SO₃ in 10 ml. H₂O.

Procedure
Centrifuge 60-ml. urine, previously adjusted to pH 4.0, with citric acid to clarify, and pipet five 5-ml. samples into 50-ml. Erlenmeyer flasks.
- Pipet 10 ml. of 0.2N sodium acetate, pH 8.5-9.0, and 5 ml. H₂O into 4 100-ml. beakers.
- Titrate 1 urine aliquot plus 10 ml. sodium acetate and 5 ml. H₂O with 2% NaOH and 6 drops of 1% alcoholic phenolphthalein to pH 8.8.
- Add NaOH in the amount indicated by the above titration to the four 10-ml. sodium acetate samples and add 2 μg./ml. of dopamine standard in 0.001N HCl to 2 of the urine aliquots.
- Prepare column by pouring 1 gm. of alumina* for chromatographic adsorption (measured in a scoop) into a water-filled column stopped at the outlet with a glass-wool plug. Stir with a glass rod. Rinse twice with 10-ml. portions of water, using vacuum. Rinse column with 5 ml.

sodium acetate, using vacuum. Discard washes. Specially constructed chromatographic adsorption columns consist of 18-mm. (O.D.) Pyrex tubing, 8 cm. long, used as a reservoir, and joined to 7-mm. (I.D.) Pyrex tubing, 10 cm. long, used for the alumina column and constricted at the outlet. The collecting receivers consist of 50-ml. graduated cylinders with double-hole stoppers, one hole for the bottom of the adsorption tube and the other for the water aspirator (vacuum).

Pour acetate from the beaker into the urine sample and add contents immediately to the column, adjusting flow rate to about 2 drops per second. If flow slows, rake the surface of alumina with a glass rod. Wash next with two 5-ml. portions of sodium acetate and then disconnect the vacuum and transfer to a new receiver. Save the old receiver contents for column pH measurement. Add 10 ml. 0.2N acetic acid, stir up alumina vigorously with a glass rod and elute by gravity and repeat with another 10 ml. of acetic acid. Detach the column when 20 ml. of eluate has been collected.

Using a pH meter, adjust eluate to pH 6.4–6.6 with 20% (w/w) NaOH.

Divide the liquid contents of each cylinder into two 15 ml. centrifuge tubes and centrifuge for 2 min.

Pipet one 4-ml. sample from each centrifuge tube into each of eight 15-ml. centrifuge tubes, four labeled “A” and four “B.”

Add 0.05 ml. iodine solution to all tubes. After 3 min. add 0.5 ml. alkaline sulfite solution to each B tube and 0.5 ml. 4.5N NaOH to each A tube. After another 3 min. add 0.6 ml. 5N acetic acid to all tubes (pH drops to about 5.3). Add 0.05 ml. H2O to each B tube and 0.05 ml. sodium sulfite to each A tube.

The fluorescence obtained is read in 5 min. and in 20 hours (having stood protected from sunlight) in a spectrophotofluorometer* by adjusting the transmission scale to 100 against a 10-μg./ml. quinine solution in 0.1N H2SO4. The wavelength settings are 320 mμ (activation) and 375 mμ. (fluorescence). Slit arrangement No. 3 and RCA 1P21 photomultiplier tube are used.

Subtract reading of A tube (blank) from corresponding B tube for dopamine value in fluorescent units. The fluorescent units for the standard are obtained by subtracting the units for the unknown from the combined units for unknown and standard.

*Amineo-Bowman, American Instrument Co., Inc., Silver Spring, Md.
Discussion

The presence of dopamine in blood has not been demonstrated (5–8). Since its presence in urine as a normal constituent is undisputed, it has been suggested that it is formed from dopa in the kidney, especially since dopa decarboxylase is present in high concentration in renal cells. The transformation of dopa to dopamine by intravenous injection of dopa has been demonstrated in man (9). However, dopa also has not been found in blood (10). The failure to find dopa and dopamine in blood does not preclude their presence but indicates the inadequacy of analytic procedures to detect them. Dopa is adsorbed by alumina and eluted by acetic acid (11), and has the fluorescence characteristics of dopamine in the present procedure (1). Its presence in urine would therefore contribute to the dopamine content. Dopa has as yet not been reported in urine (12). Shaw et al. (13) found that a substantial quantity of D-dopa was recovered from human urine after ingestion of this isomer, whereas no significant quantity of L-dopa was found after its ingestion.

Von Euler et al. (14) estimated the human urinary output of dopamine as 100–200 μg./24 hours. Drujan et al. (2) give the range as 26–595 μg./24 hours with a mean of 199 ± 36. Values for our series of subjects are listed in Table 1. In 2, the 24-hour output exceeded the Drujan range. Barbeau et al. (15) report 316 ± 15 μg./24 hours. Our mean plus standard deviation of the mean is 373 ± 63 μg./24 hours. Our results are therefore in agreement with those of Barbeau et al. It is apparent that there is considerable variation in the urinary dopamine output.

This study included 2 patients with pheochromocytoma. In the first one listed in Table 1, the urinary adrenaline and noradrenaline values before operation were 18 and 37 μg./hour, respectively. These fell to 0.55 and 3.5 μg./hour postoperatively. The dopamine values in this patient were not outside the normal range. In the other, the adrenaline and noradrenaline values before operation were 12.5 and 36 μg./hour, respectively; postoperatively they fell to 0.9 and 2.7 μg./hour. The dopamine values reported in Table 1 for this patient are clearly outside the normal range, but in the determination the dopamine reading did not increase with time, indicating that some compound other than dopamine was present and interfering. One of the criteria for determination of dopamine is the characteristic increase of fluorescence with time, which criterion is incorporated in our standard method.
Table 1. Output of Human Urinary Dopamine

<table>
<thead>
<tr>
<th>No.</th>
<th>Disease or test condition</th>
<th>Collection period</th>
<th>Range (μg/hr.)</th>
<th>Mean (μg/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (♂)</td>
<td>Normal</td>
<td>Sleep</td>
<td>5–15</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>10 (♂)</td>
<td>Normal</td>
<td>Nonsleep</td>
<td>7–38</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>6 (♂)</td>
<td>Diabetes</td>
<td>12-hour day</td>
<td>3–25</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>3 (♂)</td>
<td>Diabetes</td>
<td>12-hour night</td>
<td>4–8</td>
<td></td>
</tr>
<tr>
<td>7 (6♂, 1♀)</td>
<td>Cirrhosis of liver</td>
<td>Timed day</td>
<td>1–11</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td>7 (3♂, 4♀)</td>
<td>Parkinsonism</td>
<td>Timed day</td>
<td>3–11</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>1</td>
<td>Hypothyroidism</td>
<td>Timed day</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Thyroiditis</td>
<td>Timed day</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1 (♂)</td>
<td>Weight lifting</td>
<td>Timed during lifting</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Subdural hematoma</td>
<td>Timed day</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Hypertension</td>
<td>24-hour</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Pheochromocytoma</td>
<td>preop.</td>
<td>Timed day</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>postop.</td>
<td>Timed day</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>Pheochromocytoma</td>
<td>preop.</td>
<td>Timed day</td>
<td>70*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>postop.</td>
<td>Timed day</td>
<td>118*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>postop., 3 wk.</td>
<td>Timed day</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>postop., 5 mo.</td>
<td>Timed day</td>
<td>12</td>
</tr>
<tr>
<td>1†</td>
<td>1.5 gr. phenobarbital/24 hr.</td>
<td>Timed day</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1†</td>
<td>2.0 gr. phenobarbital/24 hr.</td>
<td>Timed day</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

*Atypical rate of formation of fluorescence.
†Hospital patient.

Barbeau (16) reported that the urinary level of dopamine could be increased in Parkinson’s disease. In our series of 7 cases the levels are within the normal range but the mean is significantly lower than the normal mean. In later studies, Barbeau et al. (15) compared the 24-hour urinary output of dopamine in a group suffering from Parkinsonism and a normal group. The mean was significantly lower in Parkinsonism. Our data confirm their observation. However, the same observations hold for cirrhosis of the liver and diabetes in our series. Since the low range of variation in the normal group falls within the range for the three diseases cited, the test could hardly be of diagnostic significance. In our series of diabetes, Parkinsonism, and cirrhosis of the liver, the patients were hospitalized; since our data indicate there is a falling off of dopamine excretion during sleep, it is possible that lack of physical exertion may to some degree account for the lower values.

While the urinary level of adrenaline falls off markedly during sleep (3), an observation which has significant physiologic implications,
dopamine levels decline far less. Noradrenaline levels hold an intermediate position.

The dopamine test has indicated significant differences between disease groups, which may be of value in substantiating or suggesting physiologic mechanisms. Its use for diagnostic purposes is limited by the large normal variation in urinary dopamine output, descending to values which approach those for which the test is inaccurate, so that individual low values cannot be established as significant. In the light of present knowledge, the significance of high values is restricted to pheochromocytoma.

References