Specific Photometric Determination of 5-Hydroxyindoleacetic Acid in Urine

Harry Goldenberg

Udenfriend's nitrosonaphthol test for 5-hydroxyindoleacetic acid (5-HIAA) is subject to interference by urinary phenolic acids and drug metabolites. Studies in our laboratory have shown that the specificity and sensitivity of the nitrosonaphthol reaction with 5-hydroxyindoles can be greatly enhanced by the addition of 2-mercaptoethanol. This observation serves as the basis of a simple, improved method for the photometric determination of urinary 5-HIAA. The 5-HIAA is extracted from urine and transferred to a colorimeter tube. Treatment with nitrosonaphthol and nitrous acid results in formation of Udenfriend's violet chromophore, which is converted to an intense blue chromophore upon addition of mercaptoethanol. Extraneous colors caused by reactive phenols and indoleacetic acid in urine are destroyed at this point by the mercaptoethanol. The intensity of the remaining blue color is proportional to the 5-HIAA concentration. Recoveries of added 5-HIAA by the proposed method are quantitative. The normal 24-hour excretion values obtained for human subjects fall in the range of 0.8 to 6.0 mg (95% confidence limits), with a mean of 3.9 mg. Drugs that produce a false positive test for intestinal carcinoid with earlier photometric methods have no effect on the new method.

Clinical studies during the last 15 years have firmly established the determination of 5-HIAA as an important diagnostic aid. 5-HIAA is a major metabolite of serotonin and is excreted in amounts that reflect serotonin production in the body. It has been estimated that approximately 90% of the serotonin is localized in the gastro-intestinal mucosa. Marked increases in the urinary excretion of 5-HIAA occur in the intestinal carcinoid syndrome (1, 2). More recently there have been reports of striking elevations of 5-HIAA excretion in bronchial adenoma (3), and of substantial increases in Whipple's disease (4) and in non-tropical sprue (4, 5). Small increases have been reported during ovulation (6), pregnancy (7), and in reactions to surgical stress (8).

The procedures available for the determination of 5-HIAA in urine include colorimetry (9, 10), paper chromatography (11), gas chromatography (12), and fluorometry (13). The colorimetric method of Udenfriend et al. (9) has enjoyed widespread use either in its original form or as a screening method (14). The analysis is based on measurement of the violet chromophore formed when 5-HIAA reacts with 1-nitro-2-naphthol in nitrous acid medium. Udenfriend et al. (15) studied the specificity of this reaction and found that of more than 20 phenolic compounds tested, p-hydroxyacetanilide was the only compound not a 5-hydroxyindole that reacted to give a violet color. Indoleacetic acid gave a red color caused by reaction with the nitrous acid. The procedure drawn up by Udenfriend et al. (9) provided for the removal of indoleacetic acid from urine by extracting it into chloroform. With this method the authors obtained normal excretion values for 5-HIAA of 2–8 mg/day (4 persons). Most extensive studies by Crout and Sjöerdsma (16) gave a normal range of 2–10 mg/day, as compared to 15–800 mg/day in malignant carcinoid. It should be noted that the normal ranges given by these authors were not corrected for 5-HIAA losses that occur during the chloroform extraction step; these losses vary from 12% to 62% (6). According to Sobel (Bio-Science Laboratories, unpublished date), normal values obtained by the Udenfriend method range up to 15 mg/day when a 5-HIAA recovery factor is included in the urine analysis.

When studied by paper chromatography, the daily output of 5-HIAA falls in the range of 1–4 mg (11). A mean excretion rate of less than 2 mg/day has been reported by gas chromatography (12). These values are lower than those obtained by the nitrosonaphthol reaction and suggest that the latter is not specific for 5-HIAA. There have been reports in the literature that a number of phenolic substances in urine interfere with the photometric 5-HIAA determination by yielding spurious colors with nitrosonaphthol (10). Serious interference has been encountered with several drugs (mephenesin, methocarbamol, and glyceryl guaiacolate) whose urinary metabolites produce a positive nitrosonaphthol reaction (10, 17).

We have noted a marked improvement in the specificity of the nitrosonaphthol reaction for 5-HIAA when 2-mercaptoethanol is added to the reaction mixture. Mercaptoethanol discharges the colors formed by phenols other than the 5-hydroxyindole derivatives. Simultaneously, the violet 5-HIAA chromophore is converted to a blue chromophore, with a two-fold increase in extinction at the new absorption maximum (645 nm). This new colorimetric reaction has been adapted to the determination of 5-HIAA in urine. The present method does not require the repetitive chloroform and ethyl acetate extractions specified in the earlier photometric methods (9, 10), nor is there any need for a preliminary dinitro-
phenylhydrazine treatment. With these simplifications the time required for the analysis has been reduced to less than 2 hours. This compares to 4–5 hours (9) and 8 hours (10) by the previous methods.

Materials and Methods

Reagents

HCl, 1 mol/liter. Dilute 83 ml of conc. HCl to 1000 ml with distilled water.

Phosphate buffer, 0.1 mol/liter, pH 7. Prepare using 2.59 g KH2PO4 and 8.31 g Na2HPO4 heptahydrate (or 4.40 g anhydrous Na2HPO4) per 500 ml solution. Refrigerate.

1-Nitroso-2-naphthol, 2 g/liter of ethanol. Store in an amber-colored bottle. See Note 1.

Sodium nitrite, 25 g/liter.

Nitrous acid. Mix 1 ml of 25 g/liter sodium nitrite with 25 ml of 1 molar HCl. Prepare just prior to use.

Mercaptoethanol, 250 ml/liter. Prepare in the hood and refrigerate.

Stock 5-HIAA standard, 250 mg/liter. Prepare in 1 millimolar HCl. Store in an amber-colored bottle and refrigerate.

Working 5-HIAA standard, 10 mg/liter. Dilute 4 ml of the stock standard to 100 ml with aqueous thiourea (1 g/liter). Store in an amber-colored bottle and refrigerate. Stable for 2 weeks or more.

Miscellaneous. Reagent-grade NaCl, ether, and ethyl acetate. The ether is freed of peroxides by shaking in a separatory funnel with one-half its volume of ferrous sulfate (50 g/liter), followed by two washes with distilled water.

Urine specimens

Twenty-four hour urine specimens are collected using boric acid as a preservative. Under these conditions the 5-HIAA is stable for a week at room temperature and for periods exceeding one month in the refrigerator.

Procedure

1. Place 5 ml of urine in a 50-ml glass-stoppered centrifuge tube or a 12-dram vial provided with a poly-seal screw cap (size No. 24). Use 5 ml water for the blank and 5 ml of 5-HIAA (1 mg/100 ml) for the standard.

2. To each tube add 5 ml of 1 molar HCl, a saturating amount of NaCl (about 4 g) and 25 ml of ether.

3. Shake the tubes for 5 min and centrifuge. If vials are used, place flat rubber cushions in the centrifuge shields or invert the standard concave cushions.

4. Transfer 20 ml of the ether extracts to clean 35 ml glass-stoppered tubes or poly-seal vials.

5. Add 4 ml of phosphate buffer. Shake for 5 min and centrifuge.

6. Transfer 2-ml aliquots of the lower aqueous layers to clean test tubes (it is convenient to use colorimeter tubes). Be careful not to carry over any ether. Do not discard the remaining aqueous solutions until the analysis is completed.

7. Add 0.5 ml of nitrosonaphthol to each tube with mixing. Add 1 ml of nitrous acid. Mix again. Incubate for 5 min at 37°C (Note 2).

8. Add 0.2 ml of mercaptoethanol to each tube. Mix. Incubate an additional 20 min at 37°C (Note 3).

9. Pipet 5 ml of ethyl acetate into the tubes. Stopper with a rubber finger cot and shake vigorously for about one-half min. Let the layers separate.

10. Read the absorbances of the aqueous (lower) layers vs. the blank at 590 nm (Klett filter No. 59). See Note 4.

Calculation

\[ \text{mg} \text{5-HIAA/24 h} = \frac{A(\text{unknown})}{A(\text{standard})} \times \frac{1}{[24-h \text{ urine volume (ml)/100]}} \]

Normal Excretion Values

The normal daily 5-HIAA excretion values for this method fall in the range of 1.8–6.0 mg (95% confidence limits) under optimum conditions of urine collection. Under average conditions, making allowance for incomplete 24-h urine collections as well as for minor aberrations in diet, a range of 1–7 mg may be regarded as normal.

Note 1

The commercial grade of 1-nitroso-2-naphthol (Eastman practical) is suitable for analytical use, although it may yield a light blank color. An improved reagent is obtained by dissolving the compound (20 g) in 100 ml of hot alcohol, adding decolorizing charcoal, and filtering through two sheets of Whatman No. 1 filter paper. The mixture is refrigerated overnight and the brown crystalline deposit filtered off on a Buchner funnel. It can be dried under atmospheric conditions (subdued light), or in a vacuum desiccator. Yield: 10–12 g.

Note 2

Formation of a deep red color in Step 7 may be indicative of the presence of metabolites of glyceryl guaiacolate (present in cough medicine) or related drugs. If upon incubation with mercaptoethanol (Step 8) the color turns a dark olive-green, comparable in intensity to the 5-HIAA standard, place the tube in a hot water bath (85°–100°C) for 5 min. Cool and proceed with Step 9. The heat treatment eliminates drug interference but does not affect the 5-HIAA chromophore, which is thermostable.

Note 3

The incubation period can be reduced to 5 min by placing the tubes in a hot water bath (use a hood).

Note 4

If A(unknown) is more than three times as large as A(standard), repeat the color development using 0.2 ml phosphate extract (from Step 6). Add 1.8 ml of phosphate buffer to the sample and proceed with
Steps 7 to 10. For the calculation, multiply the value obtained from the formula above by 10.

Development of Method

Preliminary treatment of urine. In previous methods for 5-HIAA based on the nitrosonaphthol reaction (9, 10), urine is subjected to a two-step cleanup prior to extraction of the 5-HIAA. The first step involves the addition of 2,4-dinitrophenylhydrazine to remove keto acids, which are reported to interfere with the color reaction if present in large quantity, as in rabbit urine. Human urine contains only small amounts of keto acids, however, and it is our experience that their removal is unnecessary. This is demonstrated by the quantitative recoveries of 5-HIAA obtained from untreated urine under the conditions of our method (Table 1). It is relevant to note that several laboratories now regard the use of the hydrazine reagent as optional or superfluous for the analysis of human urine (18, 19).

The second cleanup referred to above employs a double extraction with chloroform to remove indoleacetic acid from urine. This step can lead to the loss of large amounts of 5-HIAA. According to Sochor (19), Mustala (10), and other workers, the loss is dependent on the concentrations of salts present in each urine specimen. Various procedures have been suggested for coping with this problem (10, 19). In the present method the problem is circumvented by eliminating the chloroform extraction step. The indoleacetic acid and the phenolic acids that remain in the urine and are extracted into ether along with the 5-HIAA pose no difficulty, since their colored products are subsequently purged by the mercaptoethanol reagent.

From the previous discussion it is apparent that preliminary treatment of urine is not necessary. The 5-HIAA is extracted directly into ether from acidified urine. Salt is added to the urine, as in previous methods, to promote transfer of the 5-HIAA into the organic solvent.

Recovery of 5-HIAA from ether. The 5-HIAA can be recovered from the ether phase by reducing to dryness, or alternatively by re-extraction into aqueous medium. The former procedure has been used with considerable success in this laboratory. However, extended experience with urine specimens has resulted in the observation that residues may be obtained upon removal of the ether that are refractory to dissolution in water. On occasion it has been noted that some loss of the 5-HIAA standard may occur on the surface of the glass flasks used for flash evaporation of the ether. The preferred method of isolating 5-HIAA from ether is via extraction into dilute aqueous buffer. Extraction into aqueous solution serves two purposes. First, it avoids the problems attendant on evaporation. Second, by proper choice of pH the 5-HIAA may be drawn into aqueous solution unaccompanied by phenolic, non-carboxylic acids. Some compounds of this type would offer potential interference if present in large amounts during color development.

The optimum conditions for extraction of 5-HIAA from ether into aqueous buffer were carefully investigated. Phosphate solution was employed at a concentration of 0.1 mol/liter. The results are recorded in Figure 1. The extraction of 5-HIAA improved with increasing pH, reaching a maximum at about pH 6.8 and remaining constant thereafter. On this basis, a buffer of pH 7 or higher should be acceptable. According to the literature (19), 5-HIAA is unstable in alkaline solution. Phosphate buffer at pH 7 was therefore selected as meeting the criterion for efficient extraction of 5-HIAA from ether with minimal risk of decomposition. It may be noted that urinary phenols lacking an acid function are not ionized at pH 7 and remain behind in the ether layer, thereby providing a relatively clean 5-HIAA extract.

Optimum conditions for color development. The conditions chosen for estimation of the 5-HIAA in the phosphate extract provide a total volume of about 3.7 ml for the photometric reading. The concentration of the nitrosonaphthol reagent (2 g/liter)

<table>
<thead>
<tr>
<th>Sex of Subject</th>
<th>5-HIAA Recovery, %</th>
<th>5-HIAA mg/24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>94</td>
<td>3.9</td>
</tr>
<tr>
<td>M</td>
<td>101</td>
<td>3.8</td>
</tr>
<tr>
<td>M</td>
<td>92</td>
<td>5.6</td>
</tr>
<tr>
<td>M</td>
<td>96</td>
<td>4.6</td>
</tr>
<tr>
<td>M</td>
<td>98</td>
<td>2.8</td>
</tr>
<tr>
<td>M</td>
<td>92</td>
<td>3.4</td>
</tr>
<tr>
<td>M</td>
<td>95</td>
<td>3.6</td>
</tr>
<tr>
<td>M</td>
<td>102</td>
<td>3.6</td>
</tr>
<tr>
<td>M</td>
<td>90</td>
<td>4.6</td>
</tr>
<tr>
<td>M</td>
<td>97</td>
<td>3.4</td>
</tr>
<tr>
<td>M</td>
<td>99</td>
<td>3.2</td>
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<td>97</td>
<td>6.1</td>
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<td>F</td>
<td>98</td>
<td>4.2</td>
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<td>F</td>
<td>96</td>
<td>4.4</td>
</tr>
<tr>
<td>F</td>
<td>97</td>
<td>2.0</td>
</tr>
<tr>
<td>F</td>
<td>99</td>
<td>4.3</td>
</tr>
<tr>
<td>F</td>
<td>101</td>
<td>4.4</td>
</tr>
<tr>
<td>F</td>
<td>94</td>
<td>3.6</td>
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<tr>
<td>F</td>
<td>103</td>
<td>4.3</td>
</tr>
<tr>
<td>F</td>
<td>96</td>
<td>2.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>97 ± 3</td>
<td>3.9 ± 1.0</td>
</tr>
</tbody>
</table>

Table 1. Recoveries of and Normal Values for Urinary 5-HIAA (Specimens from 20 Persons)

Fig. 1. Influence of pH on the extraction of 5-HIAA from ether into phosphate buffer (0.1 mol/liter)
is not critical and may be varied from 1 to 3 g/liter. Studies of the recovery of 5-HIAA from urine indicated that somewhat better results were obtained when the nitrosoanaphthol reagent was increased in concentration from 1 to 2 g/liter. Increasing the nitrosoanaphthol to 3 g/liter failed to yield better recoveries. The choice of 3 g/liter solution would appear to be contraindicated, since significant blanks are produced at this concentration. High concentrations of nitrosoanaphthol also incur the risk of producing increased interference by phenolic acids other than 5-HIAA, although this is mitigated by the subsequent addition of mercaptoethanol.

Formation of the violet chromophore from nitrosoanaphthol and 5-HIAA proceeds rapidly and is complete in less than 5 min. Conversion to the blue chromophore progresses less rapidly, but is essentially complete in 20–30 min at 37°C or in 3 min at 100°C. To obtain these results, the second color reaction requires an excess of mercaptoethanol in the incubation mixture (0.2 ml of 250 ml/liter reagent). When small amounts of mercaptoethanol (0.2 ml of a 10 ml/liter reagent) are added to solutions containing the violet chromophore and the mixture is incubated for various periods of time, little or no alteration is observed in the violet color for periods up to one hour. After an hour there is a noticeable development of a lavender color, turning blue after 24 hours. A high concentration of mercaptoethanol (250 ml/liter reagent) serves the dual purpose of promoting rapid conversion of the violet to the blue chromophore, as well as removing spurious colors produced by phenolic acids present in the urine extract.

Conversion of the violet to blue color causes a shift in the absorption maximum from 535 nm to 645 nm (Figure 2). For maximum sensitivity the blue solution may be read at 645 nm, but at this wavelength Beer's law is not obeyed through a suitable range of 5-HIAA concentrations. Better results are obtained at 590 nm. With a No. 59 filter, the Klett colorimeter yields a linear Beer's plot through a reading of 400, corresponding to an absorbance of 0.8 (Figure 3). Excellent results have also been obtained with a Leitz Photometer at 580 nm and with a Beckman Model DU spectrophotometer at 580 nm and at 590 nm.

Results and Discussion

Recovery Studies

These studies were carried out with use of 24-h urine specimen collections. 5-HIAA was added in 50-μg amounts (0.2 ml of the stock standard) to 5-ml aliquots of urine. Water (0.2 ml) was added to the urine controls, and the specimens were then analyzed in the routine manner. As seen in Table 1, the recoveries obtained ranged from 90 to 103%, with a mean of 97 ± 3% (SD). In contrast, recoveries of 38 to 88% have been reported for the method of Udenfriend et al. (6).

During the course of these studies, 5-HIAA recoveries were evaluated with use of a phosphate buffer reagent of pH 6.3. With this reagent, the recoveries varied from 70 to 90%, but in one instance the value dropped to 60%. This result was obtained despite the fact that the 5-HIAA reference standard was processed in like manner to the urine specimen. With use of the prescribed phosphate buffer of pH 7, the recovery from the urine in question rose to 94%. It is therefore most important that urinary 5-HIAA be extracted from ether under optimum conditions of pH (Figure 1).

Specificity of the Method

The specificity of the new method was evaluated with a number of nitrosoanaphthol-positive substances that are either normally present in urine or of interest because of their high reactivity. The test compounds were dissolved in water or 10% ethanol and 5-ml aliquots were analyzed in the usual manner, i.e., extraction into ether and buffer followed by color development. The results obtained
are given in Table 2, fourth and fifth columns. Although tested at concentrations 5 to 10 times greater than the 5-HIAA standard, none of the substances yielded detectable colors or measurable colorimeter readings.

The in vitro studies reported above were supplemented by in vivo studies on two drugs (mephenesin, glycerol guaiacolate) which are reported to give false indications of carcinoid by Udenfriend's method. The subjects placed on this study were tested over a period of several days to determine their baseline 5-HIAA values. Mephenesin was then administered to three persons in the form of 2 × 0.5 g tablets ("Tolserol") per day for three days. Glycerol guaiacolate was administered to a group of four subjects in the form of cough medicine ("Robitussin") in the amount of 5 × 100 mg per day for three days. Urine collections were continued during the trial period. The urine specimens were analyzed in the usual manner except that one set of extracts was incubated with mercaptoethanol for 20 min at 37°C, while the other set was heated with mercaptoethanol in a hot-water bath for 5 min. The results are given in Tables 3 and 4. It will be noted that the mercaptoethanol treatment at 100°C completely suppressed all interference by the mephenesin and glycerol guaiacolate. At 37°C the interference by urinary mephenesin metabolites was slight. On the other hand, the treatment at 37°C did not effectively eliminate interference by glycerol guaiacolate.

We are not presently aware of any substances in urine that yield a false positive test by the new colorimeter method. 5-Hydroxytryptamine (serotonin) is present in small amounts in urine and produces a blue color reaction with the test reagents. However, as noted in Table 2, this 5-hydroxyindole derivative does not interfere with the analysis because it is not carried through the extraction procedure. A positive test by this method depends on the following structural requirements: (a) presence of a 5-hydroxyindole grouping; (b) absence of a basic substituent, such as —NH₂, which in acid medium forms an ether-insoluble salt; and (c) presence of an acid substituent (—COOH) whose ionization is suppressed in acid medium but not at pH 7.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Concn, mg/100 ml</th>
<th>Nitrosonaphthol color reactiona</th>
<th>Results by this method</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Hydroxyindoleacetic acid</td>
<td>1</td>
<td>violet</td>
<td>Klett reading</td>
</tr>
<tr>
<td>5-Hydroxytryptamine</td>
<td>5</td>
<td>violet</td>
<td>160</td>
</tr>
<tr>
<td>Indole-3-acetic acid</td>
<td>10</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>Homovanillic acid</td>
<td>10</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>p-Hydroxyphenylacetic acid</td>
<td>10</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>p-Hydroxyphenylpropionic acid</td>
<td>10</td>
<td>rose</td>
<td></td>
</tr>
<tr>
<td>p-Cresol</td>
<td>10</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>p-Hydroxyacetanilide</td>
<td>10</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>p-Methoxyphenol</td>
<td>5</td>
<td>violet</td>
<td></td>
</tr>
<tr>
<td>3,4-Dimethylphenol</td>
<td>5</td>
<td>rose</td>
<td></td>
</tr>
</tbody>
</table>

Colors produced when the indicated test compounds were incubated directly with nitrosonaphthol and nitrous acid, in the absence of mercaptoethanol.

Table 3. Influence of Mephenesin on 5-HIAA Assay by the Proposed Method

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Sex of subject</th>
<th>Baseline</th>
<th>Color</th>
<th>Klett reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. B.</td>
<td>M</td>
<td>3.7 ± 0.1</td>
<td>blue</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>L. K.</td>
<td>M</td>
<td>4.7 ± 0.2</td>
<td>blue</td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td>E. B.</td>
<td>F</td>
<td>2.7 ± 0.2</td>
<td>blue</td>
<td>4.1 ± 0.5</td>
</tr>
</tbody>
</table>

37°C C, 20 min 100°C C, 24 h

False negative results from the presence in urine of phenothiazine drugs (20) and homogentisic acid (21) have been reported with Udenfriend's method. These compounds have not been examined in our laboratory and we cannot comment on their effect under the conditions of the present analysis.

Table 4. Influence of Glycerol Guaiacolate of 5-HIAA Assay by the Proposed Method

<table>
<thead>
<tr>
<th>Urine sample</th>
<th>Sex of subject</th>
<th>Baseline</th>
<th>Color</th>
<th>Klett reading</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. K.</td>
<td>F</td>
<td>3.1 ± 0.2</td>
<td>blue</td>
<td>10.4 ± 0.8</td>
</tr>
<tr>
<td>K. C.</td>
<td>F</td>
<td>3.7 ± 0.1</td>
<td>blue</td>
<td>10.4 ± 0.8</td>
</tr>
<tr>
<td>A. K.</td>
<td>M</td>
<td>3.8 ± 0.1</td>
<td>blue</td>
<td>15.9 ± 4.0</td>
</tr>
<tr>
<td>J. B.</td>
<td>M</td>
<td>3.0 ± 0.1</td>
<td>blue</td>
<td>13.7 ± 1.5</td>
</tr>
</tbody>
</table>

37°C C, 20 min 100°C C, 5 min

Table 5. 5-HIAA Excretion Values

Table 5. The mean and upper normal 5-HIAA values cited above are compared in Table 5 with the corresponding values from the methods of Udenfriend et al. (9) and Mustala (10). It will be noted that despite the higher recoveries obtained with the new method, the
normal values are substantially lower than in the other procedures. The differences become more marked when the 5-HIAA excretion values are corrected to 100% recovery (4th and 6th columns), using the average recoveries given in Column 2 as the basis of calculation. The mean normal by Udenfriend’s method now exceeds the present method by 3.4 mg (85%); the upper normal is 3.0 to 7.3 mg (48 to 118%) higher. Values by Mustala’s method are in closer agreement with our results, differing by 1.6 mg (40%) and 1.8 mg (29%) at the mean normal and upper normal levels, respectively.

It is clear from the previous discussion that 5-HIAA methods that are based on the original nitrosonaphthol reaction suffer in varying degree from two major sources of error: (a) increased values resulting from interference by reactive phenols, and (b) decreased values resulting from low recoveries. The second effect partially compensates for the first, so that in the absence of reactive drugs the net result is a positive error of less than 4 mg 5-HIAA/24 h.

Effect of Diet

The 5-HIAA found in urine appears to be derived in large part from endogenous serotonin. However, some edible plants contain significant amounts of serotonin and their ingestion may lead to increases in the excretion of 5-HIAA. Bruce (22) has reported that pineapple juice contains 1.2–2.5 mg of serotonin per 100 ml. Walnuts were found by Kirberger and Braun (23) to contain a striking amount of serotonin (17–34 mg/100 g). Udenfriend et al. (24) analyzed a number of fruits and vegetables for their serotonin content, with the following results (expressed as mg/100 g): plantain pulp, 4.5; banana pulp, 2.8; tomato, 1.2; red plum, 1.0; avocado, 1.0, blue-red plum, 0.8; eggplant, 0.2; blue plum, 0; potato, 0; grape, 0; orange pulp, 0; and spinach, 0. Additional analyses were carried out by West (25), who failed to detect serotonin in extracts of the following foods: strawberries, gooseberries, raspberries, cherries, rhubarb, black currants, lemons, apples, figs, and prunes. From these results it would appear that walnuts, plantains, bananas, and pineapples are the principal foods that must be restricted prior to collection of urine for 5-HIAA analysis.

A brief study was made of the influence of banana consumption on the excretion of 5-HIAA. Two adult subjects were each fed two 100-g bananas and their urines collected for the subsequent 24-h period. Urine collections were also made on the days before and after the trial period. In the first subject the 5-HIAA rose from a baseline value of 3.4 mg to 10.7 mg/24 h during the trial period, and decreased to 3.2 mg on the following day. In the second subject there was a rise from 3.6 mg to 8.3 mg/24 h, followed by a decrease to 4.0 mg. The similarity of results on the first and third days suggests that the serotonin present in bananas is metabolized and excreted within 24 h. Using the results of the first and third days as the baseline, the first subject excreted 3.7 mg of 5-HIAA per banana, and the second subject excreted 2.3 mg of 5-HIAA per banana. The mean of these two values is 3.0 mg/100 g of banana. As noted in the paragraph above, 100 g of banana contains 2.8 mg serotonin, which is equivalent to 3.0 mg of 5-HIAA. This value, by coincidence, is identical to the average value obtained in our trial study and leads to the tentative conclusion that most of the serotonin present in bananas is metabolized to 5-HIAA and excreted in urine.

Comments

The blue 5-HIAA chromophore is salt-like and stable to both light and heat in the presence of excess mercaptoethanol. This combination of properties, viz., stability and water-solubility, appears to be unique to the 5-hydroxyindole structure. In contrast, all other nitrosonaphthol-positive substances that we have investigated produce colors that fade on exposure to mercaptoethanol. The decolorization is accelerated by heat. Residual pigment that may remain after mercaptoethanol treatment is readily extracted into ethyl acetate. Because the blue 5-hydroxyindole chromophore is retained in the aqueous phase, the partition is clean and provides a highly specific colorimetric assay for 5-HIAA under the conditions of the analysis.

Conversion of the violet Udenfriend chromophore to a blue chromophore is not unique to mercaptoethanol. Cysteamine also produces a blue color, but this compound is less effective than mercaptoethanol in eliminating interference from reactive phenols. Potassium thiocyanate and other sulfur compounds and reducing agents have also been tested in our laboratory. The results obtained suggest that under carefully controlled conditions, nitrosonaphthol may be useful as a reagent for the analysis of metabolites of biogenic amines other than serotonin (26).
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References