Acetaminophen Administration Interferes with Urinary Metanephrine (and Catecholamine) Determinations

To the Editor:

For three years we have been using a modification of the method of Shoup and Kießinger (1) for determination of urinary metanephrine excretion. This widely used assay involves ion-exchange chromatography of the metanephrines followed by reversed-phase “high-performance” liquid chromatography with electrochemical detection. Although in general we have been pleased with the method, occasional patients have substances in the extracts of their urine that prevent accurate determination of metanephrine. We report here that these interferences result from ingestion of acetaminophen. Although we find that acetaminophen ingestion causes less interference in metanephrine determinations by the original method (1) than with our modification, it may still introduce some error into the analysis.

Our modified method for determination of urinary metanephrines differs from the original method (1) in the following respects. After hydrolysis of the conjugated metanephrines, the urine sample (2 mL) is adjusted to pH 6.5 with NH₄OH and applied directly to the ion-exchange column. After washing the resin bed with 6 mL of water, we elute the metanephrines from the column with 4.0 mL of 2.0 mol/L acetic acid. This extract is then injected directly onto the reversed-phase column. These modifications obviate the two organic extractions in the original method and allow a smaller volume of liquid to be passed through the ion-exchange column. Hence, the modified method is somewhat faster than the original, and it routinely gives higher recoveries. Its disadvantage is that a more complex chromatogram is obtained, although metanephrine and normetanephrine can still be determined with a high degree of certainty (see retention of the boiled urine with toluene:isoamyl alcohol (3:2 by vol), according to the original method, before application to the ion-exchange column did not simplify the chromatograms. One of the “complexities” introduced into the chromatogram by this modified method is dopamine, which can be quantified simultaneously (see below).

A review of the medication records of 10 patients with either an unexplained increase in values for urinary metanephrine or an obvious interference in our metanephrine assay method revealed that nine of the 10 patients had received from 300 to 900 mg of acetaminophen daily for 24 h. In none of these patients were we able to obtain a second 24-h urine specimen, started at least 48 h after the last dose of acetaminophen. Metanephrine was determined in these specimens without apparent interference, and the values were within normal limits. Our suspicion that acetaminophen ingestion was responsible for the interference with metanephrine determinations was confirmed when two volunteers collected 24-h urine specimens while ingesting 900 mg of acetaminophen. This resulted in a large new peak on the chromatogram, with normetanephrine appearing as a shoulder on the peak. When one of the volunteers collected 24-h specimens while ingesting aspirin (1800 mg), ibuprofen (1200 mg), or codeine (60 mg) the chromatogram was not altered as compared with a drug-free collection, and urinary metanephrine excretion was normal.

To ascertain if acetaminophen also interfered with metanephrine determinations by the original method of Shoup and Kießinger (1), urine was collected while a volunteer was ingesting 1800 mg of aspirin or 900 mg of acetaminophen and extracts were prepared by both the original and the modified method. The extracts were then subjected to high-performance liquid chromatography with electrochemical detection (Figure 1). Normetanephrine and metanephrine in extracts prepared from urine collected during aspirin administration could be quantified by either the original (panel A) or the modified (panel C) method. The urine collected during acetaminophen ingestion and processed by the modified method produced a chromatogram that included major electrochemically active species, with retention times of 3.5 and 4.8 min (panel D), which were not observed without acetaminophen administration (panel C). Normetanephrine was not sufficiently resolved from these substances to allow quantification. When the same urine sample was processed by the original method, the amounts of material with retention times of 3.5 and 4.8 min were greatly diminished but the 4.8-min peak still overlapped with normetanephrine (panel B). Neither of the interfering substances was acetaminophen; this drug has a retention time slightly greater than that of 4-hydroxy-3-methoxybenzylamine (US in Figure 1), with a similar electrochemical activity, under our chromatographic conditions. Hence, the interfering substances are probably metabolites of acetaminophen. These metabolites must be relatively stable to acid hydrolysis, have a positive charge at neutral pH, and probably contain an unmodified hydroxyl group on the benzene ring. These are probably minor metabolites such as the cysteine conjugate of acetaminophen (2), which was recently shown to interfere in urinary amino acid analysis (3). These substances will also potentially interfere with urinary catecholamine determinations performed by similar methods, because the retention times of epinephrine and norepinephrine are between 2.5 and 4.0 min in our system.

Fig. 1. Chromatograms of urinary metanephrine extracts

Urine samples (24 h) from a healthy volunteer who had received either 1800 mg of aspirin (panels A and C) or 900 mg of acetaminophen (panels B and D) were prepared by the original method of Shoup and Kießinger (ref. 1, panels A and B) or the modified method described in the text (panels C and D). Extracts (200 μl) were chromatographed isocratically on an Alltech C18 column (4.6 × 50 mm, 10 μm) at 22 °C with a mixture of seven volumes of methanol and 93 volumes of a solution containing, per liter, 0.18 mol of monochloroacetic acid, 1 mmol of NaEDTA, and 15 mg of sodium sulfate, adjusted to pH 3.0 with NaOH. Flow rate, 1.5 mL/min. A Bioanalytical Systems LC-4A electrochemical detector with a glassy carbon electrode set at 0.55 V was used to detect the metanephrines. The arrow indicates the time of sample injection. Compounds were identified by their retention times relative to those of standards run separately. NM, normetanephrine; M, metanephrine; IS, internal standard, 4-hydroxy-3-methoxybenzylamine; DA, dopamine.

CLINICAL CHEMISTRY, Vol. 31, No. 6, 1985
Automated Determinations of $K^+$ and Li$^+$ with a Microprocessor-Controlled Flame Photometer

To the Editor:

I interfaced a KLInA flame photometer (Beckman Instrument Co., Fullerton, CA) to a microprocessor for automated determination of potassium or lithium ions in solution. Here I describe the design of the equipment and programming in sufficient detail to allow a clinical chemist to retrofit similar instruments. In this system, an analyst is interactively instructed in the calibration procedure and in the sample set-up. The samples are automatically analyzed, the results are stored in the microprocessor's memory, and calculations are performed, based on a calibration curve. The final results are printed in tabular form, suitable for permanent copies. The interfacing techniques described in this article can be found in many books and publications (e.g., refs. 1–6).

The primary purpose of this program is to provide detailed instructions in the calibration and operation of the instrument. In a laboratory that has frequent turnover of technicians or infrequent use of the instrument, the supervisor can spend a minimal amount for training of these procedures.

The flame photometer was equipped with a dilutor assembly for diluting lithium samples 50-fold and for diluting potassium samples 100- or 200-fold. This assembly supported a sample tray capable of holding 50 samples for auto-injection. The microcomputer, an 8-bit Model SYM-1 (Synertec Systems Corp., Santa Clara, CA), was mounted in a chassis that supported an S-100 Bus. A 16-channel, 12-bit analog-to-digital converter (Model S-100; Tecmar, Inc., Cleveland, OH), also on the S-100 Bus, was connected to the analog output of the flame photometer through a three-stage operational amplifier circuit (LM 330 OP-AMPS, available from various electronics manufacturers). The first stage was a voltage follower, the second stage had fixed gain of 10×, and the last stage had a variable gain of 10× to 50×. A low-pass filter was connected to the output of this circuit.

A hard-copy terminal (Model KSR-743; Texas Instruments Inc., Houston, TX) communicated with the microcomputer over a RS-232C data line. The terminal was used to enter experimental information and to print out instructions and tabulated data. The manual "start" switch of the flame photometer's dilutor assembly advances the sample tray one position and injects the next sample. I wired this switch in parallel with an optical coupler so that, on command from the microcomputer, the switch contact is completed remotely to start the analysis.

A program written in BASIC controls all aspects of the instrument's operation. It also instructs an analyst in the sequence of steps necessary to calibrate an assay. After calibration, as many as 18 samples are loaded onto a sample tray for automated analysis. The analyzer, data collection, and tabulation remain under control of the program until the last sample is analyzed. At this point, control of the system returns to the analyst, who selects one of several options, such as ending the program, adding another tray of samples, or starting a new experiment. The instruction set corresponding to the option selected is printed out.

Some analytical methods require that a calibration curve be used to treat the sample data to get more accurate results. This function was written into the program. After the standards are analyzed, the results are fitted to a straight line by linear regression. When the samples are tested, the raw data are first printed out and then recalculated from the slope and intercept of the linear regression; these final results are also printed out. (An example is shown in Figure 1.)

This system is suitable for several applications. The instrument was originally designed to determine Na, Li, or K ions in urine, serum, or plasma, but it has been routinely used in my laboratory to analyze the concentrations of potassium or lithium released from various medication forms during dissolution experiments. It could also be used for quantitative analysis or to assess content uniformity of pharmaceuticals containing potassium or lithium as the active ingredient.

Richard A. Soltero
CIBA-GEIGY Pharmaceuticals
Summit, NJ 07901
Current address:
Berlex Labs., Inc.
Cedar Knolls, NJ 07927

Superoxide Dismutase Activity and Zinc, Copper, and Manganese Concentrations in Leukocytes

To the Editor:

Superoxide dismutase (SOD; EC 1.15.1.1), a biologically important enzyme, appears (1) to protect cells against the potentially cytotoxic free radical superoxide anion ($O_2^{-}$). A ubiquitous enzyme, it is found in oxygen-metabolizing cells, including erythrocytes and leukocytes. Actually, the name SOD refers to a group of metalloproteins containing zinc, copper, and manganese, at least two of which appear in mammalian cells. The zinc and copper SODs are inhibited by cyanide and are found in the cytosol of the cells; manganese SOD is located in the matrix of mitochondria and is not inhibited (2). We undertook to explore the

References

Steven P. Wilson1
Dorothy L. Kamin1
Jerome M. Feldman2

Depts. of Pharmacol.1 and Med.2
Duke Univ. Med. Center
Durham, NC 27710