months at 4 °C. The material was prepared as follows:

Fresh (12-h) autopsy human tissue [skeletal muscle for MM, heart for (MM and) MS, and brain for BB isoenzyme] was homogenized at a concentration of 100 mg/ml of tris(hydroxymethyl)aminomethane hydrochloride buffer (50 mmol/liter, pH 8.0 at 25 °C, and containing sodium chloride, 100 mmol/liter and mercaptoethanol, 10 mmol/liter). The homogenate was centrifuged at 6000 x g for 10 min, applied to a column containing diethylaminoethyl-Sephadex A50, and eluted from this with buffers of increasing salt concentration (2). After elution, the eluate fractions containing the required isoenzyme (as confirmed by isoenzyme electrophoresis) were pooled, bovine γ-globulin was added to a concentration of 10 mg/ml of eluate, and the mixture was dialyzed at 4 °C for 24 h vs. three changes of tris(hydroxymethyl)aminomethane hydrochloride buffer (100 mmol/liter, pH 7.4 ± 25 °C, and containing mercaptoethanol 1 mmol/liter). Bovine γ-globulin, a convenient, inexpensive source of protein, is added to protect the enzyme from inactivation during manipulation of low-protein-concentration homogenates. After dialysis, mercaptoethanol was added to a concentration of 50 mmol/liter of dialyzed material and an equal volume of glycerol was also added. The material was stored well-sealed (plastic screw-capped, Parafilm-sealed vials) at 4 °C. Apart from stability, an advantage of such individual isoenzyme fractions is that their activities may be directly measured and appropriate dilutions used to control the sensitivity of isoenzyme procedures or as markers. I have illustrated elsewhere (3) the use of the MB preparation as an isoenzyme electrophoresis control. It is easy to combine the individual isoenzymes just before use if a marker or control mixture containing known activity of all three isoenzymes is required.

References

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Use of α-N-Benzoyl-L-arginine-p-nitroanilide as Trypsin Substrate in Estimation of αt-Antitrypsin

To the Editor:

α-N-Benzoyl-DL-arginine-p-nitroanilide (DL-BAPNA), originally developed by Erlanger et al. (1) as a trypsin substrate, is now widely used in the estimation of αt-antitrypsin (2, 3). Erlanger et al. (1) showed that purified DL-BAPNA is inhibitory and that the actual substrate is L-BAPNA. DL-BAPNA is rather insoluble; this is particularly troublesome in attempts to automate the measurement of antitrypsin, because the compound crystallizes in the tubing. Stewart (4) obtained pure L-BAPNA, found it to be much more soluble than the DL compound, and developed an automated method for measurement of trypsin inhibitors from lima bean and soybean.

We obtained pure L-BAPNA (distributed by Beckman Instruments, Inc., Palo Alto, Calif., for Protein Research Foundation, Minoh, Osaka, Japan) and confirmed Stewart’s observation that it is much more soluble than the racemic mixture. The pH/activity curve for both compounds is given in Figure 1, the conditions being those given in our previous work (3). Both have an optimum activity at pH 8.2 at 37 °C. L-BAPNA is considerably more active than the DL compound. In our previously described assay the final concentration of DL-BAPNA is 833 μmol/liter. When L-BAPNA is used in the same concentration, in assaying sera of different PI phenotypes, the ratio of activity (L/DL) is 1.88 ± 0.018 (SD). When the substrate concentrations are adjusted so that both had the same concentration of L-BAPNA, 417 μmol/liter, the ratio is 1.25 ± 0.021.

Various concentrations of L- and DL-BAPNA were tested as shown in Figure 2. Because most of the results in the literature (2, 3) were obtained with a concentration of 833 μmol of DL-BAPNA per liter, one can estimate from Figure 2 the concentration of L-BAPNA that will give the same results, namely 288 μmol/liter.

Selecting a concentration of 0.3 μmol of L-BAPNA per liter, we compared the activities of nine random sera of various PI phenotypes (6 PI MM, 2 PI ZZ, 1 PI MZ) with the activities obtained with 833 μmol of DL-BAPNA per liter. The resulting correlation line was represented by: DL = -0.03 + 0.98Lt, r = 0.9997. When the values were compared by difference, the L isomer gave an activity 79 ± 40 (SD) U/liter higher than the DL mixture, suggesting that a slightly better correlation could have been obtained had the L-BAPNA been used at the concentration estimated from Figure 2. The difference, however, is within the experimental error for individual determinations.

We had no difficulty in automating the assay of serum α-antitrypsin on the Bausch & Lomb (Rochester, N.Y.) 400 automatic system. A stock solution of L-BAPNA was prepared in the morning and assays were run both in the morning and afternoon, with use of a final concentration of L-BAPNA of 0.3 mmol/liter. Sixteen sera were assayed on each occasion. The correlation between the two times [afternoon = -0.07 + 1.064 (morning); r = 0.985] is highly significant. The control sample, assayed seven times during this period, gave a value of 3190 ± 140 (SD) U/liter.

It is obvious from Figure 2 that the substrate concentrations used did not give maximum activities. This would have been impossible to determine with DL-BAPNA, because of its low solubility. Diagnostically, the use of DL-BAPNA has been found satisfactory. The data given above permit equating values obtained with L-BAPNA to those obtained for DL-BAPNA, and would permit others to explore the establishment of optimal substrate concentra-

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**Electrical Interference with Recorders from a Paging System**

*To the Editor:*

We experienced a series of spurious pen deflections on our SMA 12/60 (Technicon Corp., Tarrytown, N. Y. 10591). These deflections, 2 to 15 mm in magnitude, occurred at seemingly random times. Since our SMA is computer coupled and reads pen position as a function of time, these deflections were severe enough to give a troublesome series of computer diagnostics. In some cases they were large enough to cause significant changes in analytical results.

We traced the problem to radio frequency interference emanating from the medical center's paging system ("Multi-Tone," 33 MHz, 300 W; Multi-  

tone Electronics Inc., New York, N. Y. 10006). The antenna is located approximately 300 yards from our installation. Once the source was identified, it became apparent that the log ratio board could be modified to stop the analyzer's responses to the activation of the paging system. Though other audio devices such as dictaphones had been known to respond to the pager, this was the first reported instrumental interference. The problem was corrected by installing capacitors across the diodes on the log-ratio board.

Because each instrument and installation is probably unique with respect to susceptibility to a specific radio frequency interference, there is little value in describing the exact electronic changes. Dr. Laessig will supply this information upon request.

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**Sources of Error in Measuring Ferritin**

*To the Editor:*

The paper by Gonyea et al. (1) very nicely pointed out the necessity of checking the purity of ferritin preparations before assuming they are adequate for use as standards in radioimmunoassay. We are prompted to describe our experience in using the method of Lowry et al. (2) to determine the amount of protein in ferritin preparations. The color yield with ferritin protein in this method is not only quite different from the color yield for an equal weight of bovine serum albumin, but is also markedly different for ferritins from different sources.

We used horse-spleen ferritin and apoferritin (from Sigma Chemical Co., St. Louis, Mo. 63178, and Miles Laboratories, Kankakee, Ill. 60901). We isolated rat-liver ferritin by the method of Penders et al. (3) from Sprague-Dawley rats that had been treated with iron to induce ferritin formation (4).

The true protein content of the ferritin solutions was determined by digesting the protein with H2SO4/H2O2 in tubes in a heating block at 300 °C and measuring the resulting ammonia by the Folin phenol reagent. *J. Biol. Chem.* 193, 265 (1951).


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