letters

More Practical Modification of a 5'-Nucleotidase Assay

To the Editor:

We have been using the excellent method of Dooley and Racich (*Clin. Chem.* **26:** 1291, 1980) for 5'-nucleotidase (EC 3.1.3.5) since our original paper describing essentially the same method was declined by *Clinical Chemistry* in February 1979. We found that measurement at 293 nm of the uric acid produced in this reaction was not practical in a clinical laboratory. The Beckman DB or DU or Varian spectrophotometers are too cumbersome and a 300 N or Stasar Gilford spectrophotometer or standard GEMSAEC do not provide for readings at that wavelength. Instead, the uric acid can be assayed by using a peroxidase reaction, with either phenol and 4-aminoantipyrene or 3-hydroxy-2,4,6-triiodobenzoic acid as indicator.

If phenol, 4-aminoantipyrene, and peroxidase (EC 1.11.1.7) are added to the working substrate immediately before the serum sample (final concn. 14 mmol/L, 1.3 mmol/L, and 195 U/L, respectively) and the incubation time is extended to 13 min, the absorbance can be read at 500 nm. If a shorter incubation time is desired, 3-hydroxy-2,4,6-triiodobenzoic acid (Calbiochem-Behring, La Jolla, CA), final concn. 3.5 mmol/L, can be used in place of the phenol and 4-aminoantipyrene, and readings taken at 520 nm. Actually, use of the latter indicator is better because it is more sensitive and because phenol can easily denature the proteins.

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Uricase Immobilized on Walls of Glass Tubes Used as Reactors in Continuous-Flow Analyzes

To the Editor:

We report a combination of techniques that were independently developed in the areas of chromatography and enzyme engineering, resulting in the preparation of open-tube wall reactors containing immobilized enzyme. Use of these reactors in mixing-delay coils is ideal for segmented or unsegmented continuous-flow analysis of chemical species of clinical interest. Their potential application, however, extends throughout the province of chemical bonding of proteins to a silica framework.

In 1978 we showed that, if the enzyme is relatively inexpensive and relatively stable, so that it retains reasonably constant activity toward the substrate at room temperature, it can be directly used in solution for repetitive determinations in closed-flow systems (*1*). Unfortunately, important clinical determinations involve enzymes that are not sufficiently stable or inexpensive to be used in solution for relatively long periods of time or at the high activities required for successful closed-flow systems. Enzyme immobilization offers an attractive alternative in the form of wall reactors for use in open- or closed-flow systems, with or without air segmentation. "Whisker" surfaces in glass capillary gas chromatography were introduced in 1975 by Pretorius et al. (*2, 3*). Sandra et al. (*4*) have recently reviewed the advantages (from the viewpoint of gas-chromatographic analysis with glass capillary columns) of whiskers grown on the walls of the capillary column. Silica "whiskers" (filaments) formation is a rather easy and reproducible procedure for greatly increasing surface area. Gaseous hydrogen fluoride has been the most commonly used reagent for roughening glass surfaces; the technique results in filamentary protrusions (whiskers) of nearly perfect structural form. However, with hydrogen fluoride in gas form, whisker growth is non-uniform and the gas is not easily handled. Onuska et al. (*5*) provided an attractive alternative, the use of ammonium hydrogen fluoride at high temperatures. We have applied their method to grow whiskers on the walls of borosilicate glass tubing coiled for use in continuous-flow analyzers with the results shown in Figure 1.

Because of the success we have had in immobilizing uricase (EC 1.7.3.3) on controlled-pore glass chips, we have applied the technique of Robinson et al. (*6*), with slight modification, to chemically immobilize the enzyme directly on the "whiskers." In brief, the silica framework of the coil inner wall is converted to aminoalkylsilane glass by reaction with an acetone solution of 3-amino-propyltriethoxysilane, glutaraldehyde is attached to the aminoalkylsilane glass, and the enzyme is attached to the glutaraldehyde.

Immobilization of uricase by this procedure and on controlled-pore glass has provided preparations retaining about 70% of their original activity, even after 10 months (*7*). Uricase-containing

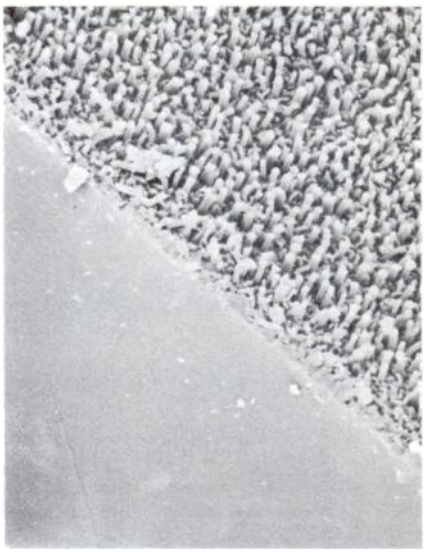

Fig. 1. Electron-scan micrograph of silica filaments on the walls of wide-bore Pyrex glass tubing

Original magnification: 600×. The photograph clearly shows the contrast between the treated and untreated surface.

31-turn coils (wide-bore Pyrex glass, 2 mm i.d., 9 mm o.d.) were used to determine uric acid in human blood serum. The coil was incorporated into a custom-assembled closed-flow, unsegmented unit consisting of a three-electrode amperometric detector with auxiliary electronics assembled with modular units from the MP-1000 system (Pacific Precision Instr., Concord, CA), a Sargent SRL recorder, and a Masterflex peristaltic pump (SRC Model 7020 speed controller and 7014 pump head). The sample was injected with the help of a gas-tight syringe and a PB600-1 repeating dispenser (both from Hamilton Co., Reno, NV 89510). Typical reservoir solutions were 200-mL volumes of a borate–ammonium sulfate buffer of pH 9.4, 0.10 mol/L in ammonium and borate, containing 2×10^6 U of catalase (EC 1.11.1.6; Sigma Chemical Co., St. Louis, MO 63178).

The continuous-flow system afforded the processing of over 200 samples per hour at a gravitationally controlled flow rate of 21 mL/min and in the concentration range of 1 to 100 mg of urate per 100 mL of solution, with only 1.05 U of uricase immobilized on the wall of the coil. The open nature of the reactor type we describe allows easy flow of solution by gravitational action and produces more convenient flow characteristics than does the forced-flow operation required with packed-type reactors (*7*).

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