Then their contents were assayed for vitamins E and A.

Our results all agreed, within the error of the methods, showing that neither Venojet nor Vacutainer Tube stoppers of the types available in South Africa contain dyes or antioxidants interfering with the assay of vitamin E, in agreement with the work of Sinclair and Slattery (1), or with the assay of vitamin A.

Although these results indicate that the particular products we examined are suitable for use in the collection of blood for fluorometry of vitamins A and E, we think that anyone undertaking fluorometric investigations would be well advised to test any rubber that comes into contact with their samples or, where practicable, to replace rubber with plastic, glass, or polyurethane.

References

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Lack of Effect of 2,3-Dimercaptopropanol on Activation of Plasma Renin Precursors

To the Editor:

Because of the increasing interest in active and inactive renin, we investigated the effect of 2,3-dimercaptopropanol on the activation of plasma renin precursors. N-Ethylmaleimide reportedly inhibits the conversion of high molecular weight inactive renin to the active form of lower molecular weight during extraction from porcine kidneys (1), and it has been used as an inhibitor in investigations of active renin (1–3). This is in harmony with the suggestion that conversion of renin into its active form is catalyzed by an agent(s) requiring free sulfhydryl groups, which N-ethylmaleimide blocks (1).

On the other hand, 2,3-dimercaptopropanol ("British anti-Lewisite") is a well-known agent for cleaving disulfide bridges (and thereby liberating free sulfhydryl groups) and has been widely used in estimations of plasma renin activity (PRA), as part of a system of inhibitors preventing angiotensin I degradation (4–9). We wondered if PRA assays that include dimercaptopropanol measure both active renin and activated renin precursors.

Plasma samples from seven normal men were pooled, and portions were dialyzed at pH 4.5, then incubated for 30 min at 32 °C before being dialyzed to pH 7.4. This treatment provides an alternative method of inactivating enzymes that degrade angiotensin I (10). We incubated plasma buffered to pH 6.0 for 90 min at 37 °C and calculated PRA from the rate of angiotensin I generation during this period. This rate was linearly related to time. Duplicate measurements of PRA in dialyzed plasma with and without dimercaptopropanol gave mean values of 12.8 and 11.3 nmol/L per hour, respectively. The mean value for undialyzed plasma was 12.8 nmol/L per hour. By Student's t-test, these results were not significantly different.

This suggests that in patients with normal PRA values, dimercaptopropanol in the concentration we used does not activate any renin precursors that may be present. In most methods for PRA it is doubtful if renin precursors are activated. An attempt to activate renin by simple acidification to pH 2.5 failed to show any increase in PRA but instead a decrease, probably owing to substrate inactivation at this low pH.

References


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Creatine Kinase Isoenzyme MB: Discrepancy between Immuno-inhibition and Electrophoretic Measurement in Lyophilized Proficiency Samples

To the Editor:

We recently examined a series of five creatine kinase (CK; EC 2.7.3.2) samples circulated by the Center for Disease Control (CDC), Atlanta, GA, as part of a proficiency testing program. These samples consist of human heart homogenate added to a Tris/bovine serum albumin/N-acetylcysteine matrix (NAC) and lyophilized. They contain both CK-MM and -MB isoenzymes.

Using the Boehringer Mannheim CK MB-activated reagent kit, we found values for total CK in this reconstituted material that agreed well with values issued in a preliminary report to participants in the program. However, on attempting to assess the MB content of the material we obtained discrepant values for the MB isoenzyme determined by immunoinhibition as compared with electrophoresis. The preliminary CDC report did not state the percentage MB composition of the material, but did indicate it to be constant in each of the five samples.

When determined by the immuno-inhibition method with use of the Merck CK-MB NAC-activated kit, the percentage MB activity in each sample was constant, about 40%. The Merck reagent includes antibody to inhibit M subunits, and it is assumed that all M subunit activity is inhibited, that only B subunit activity remains, and that B subunit activity remaining in the sample generally derives from CK-MB.

When the same samples were examined by cellulose acetate electrophoresis with fluorescence scanning (detection limit, 3 U/L at 30 °C), only CK-MM and -MB activity were observed and the percentage MB was again constant, but only about 11%. Visual inspection of the electrophoretic strip confirmed its proportion to be small as compared to MM.

To resolve the discrepancy, we further examined the samples after separation on DEAE-Sephadex, using the