


7. Meiatini, F., Complexo di reagenti per la determinazione enzimatica del glucosio (sistema glucosio ossidasi/perossidasi) in metodo manuale e automatico, con lettura a termine o in cinetica. Italian Patent no. 986883 (1975).


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No Ultrafast Alkaline Phosphatase Isoenzyme?

To the Editor:
We would like to amend and clarify our conclusion and summary in our abstract, no. 098, Clinical Chemistry 25: 1082, 1979. From our initial three random cases we concluded that there was suggestive evidence consistent with the presence of an ultrafast isoenzyme. However, it was the ultrafast fraction or band, which may or may not have indicated the presence of isoenzyme activity in the terminal anodal region, that we saw most commonly in our early limited study associated with neoplastic diseases of the liver. On reappraising our initial experimental and control plates containing the randomly selected cases, although the plates had probably faded somewhat, faint blue or blue-violet bands were seen in most of the terminal anodal band regions in the former but not the latter plate. We also detected an inadvertent technical error which led to a misassignment of the control plate origin. The terminal absorbing bands in the control plate should align with the ultrafast band region in the experimental plate containing the randomly selected cases.

Our subsequent studies have on occasion suggested the existence of an ultrafast band with enzymic activity on prolonged comparison of subtle differences in staining intensity between the control and experimental plates. However, we have found that the experimental design lacks consistent reproducibility on repetitive testing of the same samples. This may be related to the potential differential liability of the diazonium staining reagent between the control and experimental plate, which could have randomly biased the results of our initial and subsequent studies.

In conclusion, we are unsure of the definite existence of an ultrafast isoenzyme. We feel the ultrafast band or fraction has a nonspecific etiology in that it may be found in patients with benign and malignant liver disease irrespective of the bilirubin concentration. It may well be an artifact of the diazonium staining reagent, Fast Blue RR, and bilirubin, as previously indicated in the literature. We are now of the opinion that a more sophisticated approach is needed, possibly an immunologic technique, to clarify the phenomenon of an ultrafast band.

The opinions or assertions herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Defense or the Department of the Navy.

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Monitoring Serum Thiopental Concentrations by Liquid Chromatography

To the Editor:
A simple liquid-chromatographic method for determining serum thiopentals was developed in our laboratory because concentrations of this drug in blood needed monitoring during cerebral resuscitation.

Several such methods, involving various solvents and extraction techniques, have been published for thiopental (1–3). However, we elected to make minor modifications to the conditions we currently use for monitoring anticonvulsants. This approach shortens the switching time between analyses to a minimum, and thiopental samples can be prepared in the same manner as anticonvulsant samples.

Our chromatograph is a dual-pump Hewlett-Packard 1084A (Hewlett-Packard Co., Palo Alto, CA 94304) equipped with a 1030A variable-wavelength detector set at 280 nm and an automatic sample turntable. The column (10 × 0.46 cm) and guard column (3 × 0.46 cm) both contain Spheri-5, C-18 reversed-phase packing. These were obtained as the MPLC cartridge system from Brownlee Labs, Santa Clara, CA 95050. The solvent flow rate is 2 ml/min at 55 °C.

The mobile phase used for thiopental is a 4/1 (by vol) mixture of our anticonvulsant mobile phase (pump A) and methanol (pump B). The anticonvulsant mobile phase is 35/65 (by vol) methanol/phosphate buffer (5 mmol/L, pH 6.5); consequently, the methanol and buffer contents of the thiopental mobile phase are 480 mL/L and 4 mL/L, respectively.

Protein in the samples is precipitated with two volumes of acetonitrile. After centrifuging, the supernate is directly injected into the chromatograph. This technique is the same one we use to prepare anticonvulsant (4), o xo-barbiturate, and theophylline samples for chromatography.

Figure 1 shows a chromatogram of thiopental in deproteinized serum.

The absolute recovery of thiopental from serum was calculated by analyzing blank serum supplemented with 12.5 mg of the drug per liter. The averaged result (n = 5) was 12.4 (SD 0.43) mg/L, com-