LETTER TO THE EDITOR

**USE OF TRICINE BUFFER FOR ENZYME HISTOCHEMICAL
DEMONSTRATION OF ALKALINE PHOSPHATASE
ACTIVITY BY LEAD CITRATE METHOD**

We already reported two new histochemical methods for alkaline phosphatase (ALPase) activity; by replacing Tris buffer used in the original lead citrate method (3) with either glycine buffer (5) or 2-amino-2-methyl-1-propanol buffer (6), we could obtain finer reaction products and/or more sensitive detection. Recently tricine (N-Tris (hydroxymethyl)-methylglycine) buffer has been used for the demonstration of *p*-nitrophenyl phosphatase activity and has given fine reaction products (2, 4). We therefore tested tricine buffer for ALPase and reported the results below.

Adults Wistar rats were perfusion-fixed with 0.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, containing 8% sucrose for 10 min. Small pieces of the liver and kidney were rinsed, sliced unfrozen to about 40 μ m in thickness by a Microslicer (Dosaka EM, Kyoto) and incubated for 40 min at 37°C in various solutions for ALPase demonstration: tricine buffer of 5, 10, 25 and 50 mM was used instead of 28 mM Tris (3); β -glycerophosphate was either 5 or 20 mM; other ingredients were 3.9 mM magnesium sulfate, 2.0 mM lead citrate and 8% sucrose; pH was adjusted to 9.0. Osmicated specimens were dehydrated, embedded in Spurr's resin and observed electron microscopically with light counterstaining by uranyl acetate.

The precipitate of lead citrate formed by alkaline phosphatase activity was observed in the same location as the original lead citrate method (3) no matter which concentration of tricine was used for the incubation solution: on the bile canalicular membrane of hepatocytes (Figs. 1, 2) and on the microvillar membrane of renal proximal convoluted tubular epithelia (Fig. 3). The size of the lead precipitate was coarse with 25 and 50 mM of tricine in the medium but became minute at 5 and 10 mM of the buffer concentration. The concentration of the substrate, β -glycerophosphate, did not affect the result notably.

The present results show that tricine buffer can be used for the histochemical detection of ALPase successfully. The reaction product, lead phosphate, was fine with the lower concentration of tricine in the incubation medium, which corresponds well to the results with glycine buffer (4). Tricine, which has three glycine residues in a molecule, most likely shares the characteristics of glycine as a chelator. Probably at 5–10 mM, tricine has the most favorable chelating strength to give a fine lead precipitate. Combining our results on ALPase with others on *p*-nitrophenyl phosphatase, the versatility of tricine buffer as a vehicle of a histochemical incubation medium is apparent. Because of their strong buffering capacity, wide effective pH range, and low toxicity to biological phenomenon, tricine as well as other Good buffers (1) can be a choice for various histochemical solutions.

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FIGS. 1, 2. ALPase activity in the rat liver revealed by the medium buffered with tricine. Fine precipitates are seen on the bile canalicular membrane in both cases.

FIG. 1. The medium contains 10 mM tricine and 20 mM β -glycerophosphate. $\times 27,000$

FIG. 2. The medium contains 5 mM tricine and 5 mM β -glycerophosphate. $\times 27,000$

FIG. 3. ALPase activity in the rat kidney revealed by the medium containing 5 mM tricine and 5 mM β -glycerophosphate. Lead precipitates are seen along the microvillar membrane of the proximal tubular epithelium. $\times 27,000$