

# Measurement of alkaline phosphatase of intestinal origin in plasma by P-bromotetramisole inhibition

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## Abstract

**L-p-bromotetramisole was used to inhibit non-intestinal alkaline phosphatase (of liver or bone origin) (EC 3.1.3.1; ALP) in plasma, and intestinal ALP was measured from the uninhibited activity. The method of determination is convenient and correlated well with measurement by immunocapture assay. If carried out in parallel with wheat-germ lectin precipitation of bone ALP, subtraction of intestinal ALP activity from that of non-bone ALP in the supernatant can be used to measure the ALP that originates from the liver in men and non-pregnant women.**

A simple procedure for the separation and quantification of bone and non-bone alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum) EC 3.1.3.1; ALP) in plasma, using wheat-germ lectin to precipitate the bone fraction, has been described.<sup>1,2</sup> The non-bone supernatant fraction includes liver and, when present, ALP of intestinal origin. For quantification of the liver fraction, therefore, intestinal ALP must be measured separately and subtracted from the non-bone activity. We used densitometric scanning of ALP separated by cellulose acetate electrophoresis for this purpose.<sup>3</sup> This is able to show intestinal ALP of  $\beta$ -globulin mobility, and, after neuraminidase pre-treatment, a more anodal so-called "intestinal-variant" fraction,<sup>4,5</sup> both of which are thought to be of intestinal origin. Electrophoresis is, however, inconvenient, and this detracts from the simplicity of the precipitation procedure.

We have also used a monoclonal antibody reacting with both intestinal and intestinal variant ALP in an immunocapture assay to quantify intestinal origin ALP.<sup>5</sup> Once again, however, the protracted determination time is a disadvantage. Using purified human isoenzymes, L-p-bromotetramisole inhibits the bone and liver "tissue-unspecific" forms of ALP with minimal inhibition of the intestinal isoenzyme.<sup>6</sup> L-p-bromotetramisole inhibition can provide a convenient procedure for the measurement of intestinal ALP in plasma. Though correlation with other inhibitor techniques has been reported, however,<sup>6</sup> comparisons with more direct methods of measuring intestinal origin ALP are few. We therefore compared measurement of intestinal origin ALP in plasma by L-p-bromotetra-

misole inhibition with its immunological determination.

## Methods

We measured ALP activity in plasma in accordance with the International Federation of Clinical Chemistry (IFCC) recommendations,<sup>7</sup> but at 37°C, using 4-nitrophenylphosphate as substrate and 2-amino-2-methyl-1-propanol as buffer, and with reagents from Boehringer Mannheim Ltd, Lewes, East Sussex. Determinations were made on the Encore analyser (Baker Instruments Corporation, Allentown, Pennsylvania, USA).

For the inhibitor studies, (L-p-bromotetramisole oxalate (Aldrich Chemical Company Ltd, Gillingham, Dorset) was added to the substrate solution at a concentration of 0.1 mmol/l. ALP activity was determined in the presence and in the absence of inhibitor, and the percentage inhibition determined.

Intestinal origin ALP in plasma was quantified immunologically as described previously<sup>5</sup> using monoclonal antibody to human intestinal ALP (a gift from the Imperial Cancer Research Fund Laboratories). For this, the antibody was coated on to microtitre plates and used to extract intestinal origin ALP from plasma by overnight incubation at room temperature. After plate washing ALP substrate was added and intestinal ALP activity measured from the colour development following a further two hours of incubation at 37°C. Intestinal origin ALP activity was calculated from a standard curve using standards of activity 2–20 U/l, prepared by dilution of ALP extracted from human small intestinal mucosa. The standard curve was linear up to at least 20 U/l. Samples with activities above this range were appropriately diluted with saline. Interbatch (n = 19) imprecision was less than 6% at 20 U/l. The detection limit for intestinal origin ALP was 0.5 U/l. Using sera containing high concentrations of bone, liver, and placental ALP, cross reaction of the assay with these fractions was less than 0.2%.

The effect of the inhibitor on intestinal ALP was studied using the ALP from small intestine, which was added to plasma previously heated at 65°C for 15 minutes to destroy endogenous ALP, and its ALP activity was measured with and without inhibitor. To assess the inhibition of endogenous bone and liver in ALP plasma, five samples showing only the bone or liver fractions, but no detec-

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table intestinal origin ALP on electrophoresis (detection limit 3 U/l) and immunological assay, were examined with and without inhibitor.

Ninety four plasma samples of total activity up to 780 U/l submitted to the diagnostic laboratory and showing intestinal ALP by electrophoresis and immunological techniques were examined with and without inhibitor. Intestinal ALP by the inhibition method was compared with that determined immunologically.

### Results

Intestinal ALP added to plasma to an activity of 330 U/l was inhibited by 8%. The plasma samples (total activities between 340 and 640 U/l) containing only liver and bone ALP showed an average inhibition of 95.7% (range 95.4 to 95.9%) by L-p-bromotetramisole. The activity of intestinal origin ALP was therefore calculated as:

$$\frac{B - 0.04T}{0.88}$$

where T = total ALP activity and B = activity in the presence of L-p-bromotetramisole.

Intrabatch (n = 19) imprecision in the presence of inhibitor gave a coefficient of variation (CV) of 7.5% at a mean ALP activity of 23.7 U/l. The interbatch (n = 19) CV was 9.1% at a mean activity of 25.8 U/l.

Good correlation was obtained between activity by the L-p-bromotetramisole inhibition method (y U/l) and by immunoassay (x U/l). A correlation coefficient (least squares method) of 0.96, regression equation  $y = 1.16x - 2.5$ ,  $S_{yx} = 8.11$ , was obtained on the 94 plasma samples with an intestinal origin ALP activity of up to 185 U/l.

### Discussion

Measurement of intestinal origin ALP in plasma can be made rapidly and conveniently by L-p-bromotetramisole inhibition and correlated well with direct determination by immunoassay. The inhibitor assay provides a suitable procedure for assessing the intestinal origin ALP content of plasma before wheat-germ lectin precipitation. The latter separates plasma ALP into bone and non-bone fractions. The non-bone fraction is principally of liver origin, but intestinal origin ALP up to an activity of 20 U/l may be anticipated in about 20% of healthy plasma samples, with even higher activities in disorders such as hepatic cirrhosis, chronic renal failure, and diabetes mellitus.<sup>8</sup> This makes measurement of the

intestinal contribution essential if the activity of the liver fraction is to be individually quantified. In men and non-pregnant women activity remaining after subtracting intestinal origin ALP activity from that of the non-bone fraction may be regarded as exclusively measuring ALP of liver origin, though the non-bone fraction may rarely contain non-precipitated immunoglobulin-bound ALP (present in less than 0.5% of patient plasmas<sup>9</sup>) or very low activity (generally less than 1 U/l<sup>10</sup>) of placental-type ALP in malignancy. In mid and late pregnancy placental ALP contributes significantly to the non-bone fraction after lectin precipitation, and because it is also resistant to L-p-bromotetramisole inhibition, this cannot then be used to quantify the intestinal fraction, though, as shown by Mazda and Gyure,<sup>11</sup> theophylline inhibition of non-placental ALP can be used to quantify the placental contribution.

In conclusion, p-bromotetramisole inhibition provides a convenient procedure for the measurement of intestinal origin ALP in the plasma of non-pregnant women and correlates well with measurement by immunocapture assay. If carried out in parallel with wheat-germ lectin precipitation of bone ALP, subtraction of intestinal origin ALP activity from that of the non-bone ALP in the supernatant can measure the ALP that originates from the liver.

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