Plasma pseudocholinesterase is responsible for the metabolism of ester local anesthetics. In most individuals, the metabolic rate is rapid and the risk of overdosing is small. However, there are genetic variants in which the enzyme is comparatively inactive and thus these individuals are at greater risk than those with typical enzyme. This paper reports enzyme kinetic parameters for hydrolysis of 2-chloroprocaine by typical and atypical pseudocholinesterase in an effort to quantitate the difference between the typical and atypical enzymes.

METHODS
All measurements were accomplished using a Beckmann Model 25 spectrophotometer with the slit width on the three times normal setting. Venous blood was obtained from nine volunteers; three typical, three heterozygous atypical and three atypical. The blood was allowed to clot and then centrifuged for ten minutes. Stock solutions of 2-chloroprocaine hydrochloride (325 μM) were prepared daily for use in the hydrolysis studies. The sample cuvette contained 0.10 ml serum; 0.80 ml Sorensen’s phosphate buffer, pH 7.40; varying concentrations of 2-chloroprocaine (3.7-37 μM) and enough saline to bring the final volume to 1.0 ml. The reference cuvette was prepared identically, except saline was substituted for substrate. 2-chloroprocaine hydrolysis rates were derived by following the disappearance of substrate as a function of time. K_m and V_max for three volunteers of each genotype were derived from double reciprocal plots according to the method of Lineweaver and Burke. All Lineweaver Burke plots were examined for linearity using standard least squares regression analysis.

RESULTS
K_m and V_max for 2-chloroprocaine hydrolysis by the three genotypes are shown in the table. V_max was the same for each genotype. Typical and heterozygous plasma cholinesterase had similar K_m values while the K_m values for the atypical enzyme were significantly greater and showed a greater variation. Hydrolysis rates for 2-chloroprocaine at fixed, high substrate concentrations were significantly less than predicted by the enzyme kinetic parameters, suggesting substrate inhibition. The figure is a representative double reciprocal plot over a wide range of substrate concentrations. The hyperbolic shape of the curve is typical of classical substrate inhibition.

DISCUSSION
Kalow and Hersh, et al., reported that the functional difference between typical and atypical enzyme was in affinity (K_m) rather than catalytic rate (V_max). The data for 2-chloroprocaine hydrolysis further support this hypothesis. There was no biologically significant difference in V_max among the genotypes studied. K_m for atypical enzyme was thirteen times typical; K_m for heterozygous enzyme was two times the

K_m for typical. V_max with heterozygous serum was slightly and significantly greater than with either typical or atypical serum. However, the biological significance of this finding probably small and may, in fact be artifactual.

The substrate inhibition of both typical and atypical plasma cholinesterase was first suggested by Kalow for procaine. The results reported here confirm that finding with 2-chloroprocaine. 2-chloroprocaine serum concentrations in the clinical situation approximate K_m, and thus one would not expect substrate inhibition to be of any clinical significance, except perhaps in the case of an overdose.

REFERENCE