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*In reply:*—We were pleased to learn that we are not the only laboratory using an enzymatic method for the assay of cholinesterase with succinylcholine as a substrate. Because our kinetic approach is more practical than assaying in stages, however, we would like to provide further clarification of our procedure.

The use of water in the reagent blank instead of serum does not contribute to any serious error due to nonspecific hydrolysis by "substances other than cholinesterase." This is because hydrolysis of both succinylcholine and succinylmonocholine is catalyzed by the same enzyme, *i.e.*, cholinesterase,<sup>1</sup> and because only a small fraction of the succinylcholine present (2-6% of 0.5  $\mu$ mol) is hydrolyzed to succinylmonocholine. The enzyme is therefore acting mainly on the dicholine ester during the first 10 min of our assay. The contribution to the rate of choline formation by succinylmonocholine is therefore negligible because it is hydrolyzed more slowly than the dicholine ester.<sup>1</sup> Evidence that nonspecific hydrolysis of succinylcholine is not serious can be seen by the extremely low activities of the  $E_1^a E_1^a$  and  $E_1^s E_1^s$  genotypes.

Phenol and, especially, aminoantipyrine are indeed inhibitory. The latter, at the concentration we use, produces a 20% inhibition of cholinesterase when assayed by the propionylthiocholine method. The concentrations we use are optimal, *i.e.*, activity decreases at higher and lower concentrations.\* If the conditions for assay are consis-

tently adhered to, the inhibitory effect of the dye may be disregarded because it would be the same for low as well as high enzyme activities. We have obtained a linear relationship between activity and enzyme concentration up to 250 U/l, and this should provide ample validation of the test. (Here, we apologize for an error in our paper. On page 510, second column, third paragraph from the bottom, please read 250 U/l instead of 25 U/l.)

We agree that the nondeliquescent choline iodide would be preferable to choline chloride for calibration. The calibration itself is necessarily an endpoint assay as can be seen in figure 2 of our paper: in the absence of cholinesterase there are no steady-state rates to measure. The reaction is virtually complete in 3 to 4 min. We wait at least 10 min before taking the absorbance reading.

Differences between the "average" cholinesterase activity of a population, or reference value, are bound to occur between laboratories. We recommend that every laboratory establish its own reference values.

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