of the reducing reagent we describe as an alternative to the phosphorus reducing reagent supplied by Pierce Chemical Co. for the semimicro-scale assay of phosphorus in serum.

References

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Inhibition Inversion in the AACC Selected Method for Serum Cholinesterase

To the Editor:
I was recently asked to do cholinesterase phenotyping on two patients who had already been phenotyped by the coupling of propionyl-thiocolinesterase to the Ellman reaction and using dibucaine and fluoride as inhibitors (1). The method employed in this hospital involves a 50 μmol/L benzoylcholine substrate, and activity is measured at 25°C. The percentage inhibition of the enzyme by 10 μmol/L dibucaine and 50 μmol/L fluoride are measured by the analytical scheme of King (2) and King and Griffin (3).

Both methods gave the same phenotypes for the patients, but the referring laboratory asked why the phenotype E1/E1 gave practically the same percentage inhibition by dibucaine for both substrates, yet remarkably different percentage inhibitions on using fluoride. With benzoylcholine as substrate, the fluoride number of the E1/E1 phenotype was 25 and with propionylcholine as substrate it was 82. For an E1/E1 phenotype the fluoride number with benzoylcholine as substrate was 57 and with propionylcholine it was 79. The fluoride numbers of the usual and atypical phenotypes overlapped when propionylcholine was the substrate, but were much more clearly separated when benzoylcholine was used.

In the Selected Method of the AACC (4) in which propionylcholine is the substrate, it is appreciated that temperature has a large effect on fluoride inhibition. What does not appear to have been appreciated is the bizarre inhibitor results obtained with sodium fluoride. The late Dr. J. King found the degree of inhibition for all phenotypes to increase with the chain length of the substrate (5). He also noted that at lower inhibitor concentrations the relative phenotype inhibitions with propionylcholine are the reverse of that found with benzoylcholine as substrate and that this becomes inverted with increasing concentration of fluoride. King further investigated the inhibitory effects of various concentrations of fluoride on the hydrolysis of propionylcholine by several cholinesterase phenotypes (Figure 1). Other workers either did not use sufficiently high concentrations of fluoride in phosphate buffer, or conducted their experiments at 25°C rather than 37°C and thus did not appreciate this reverse inhibition. Garry (6) did use concentrations of fluoride up to 10^-2 mol/L, but in Tris buffer.

The Selected Method recommends a reaction concentration of 2 mmol/L propionylcholine substrate, 4 mmol/L sodium fluoride, phosphate buffer (pH 7.6), and a reaction temperature of 37°C. These conditions yield the highest percentage inhibitions but the percentage inhibitions for the usual (78-81%) and atypical (82-86%) homozygotes and the usual and atypical heterozygotes (78-82%) all overlap. A fluoride-resistant homozygote gives a 54% inhibition, one patient heterozygous for the fluoride and silent genes is quoted as having 65% inhibition, and a range of 67 to 69% inhibition is given for the atypical and fluoride-resistant heterozygote.

King commented that these conditions chosen are close to the point of inhibition inversion, and incredibly in an official Selected Method this would appear not to have been appreciated.

References
4. Dietz, A. A., Rubinstein, H. M., and Lu-

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The authors of the paper in question respond as follows:

To the Editor:

In reply to the Letter of Dr. McQueen, who is disturbed that $E_i^1 E_i^1$, $E_i^1 E_i^2$, and $E_i^2 E_i^2$ variants of cholinesterase all give the same fluoride inhibition in the Selected Method: the answer is indeed simple. We selected the fluoride concentration to do exactly that, since we believed it would be easier to interpret results if reduction of fluoride inhibition always indicated the presence of $E_i^1$.

The choice of fluoride concentration used in our method is based on the results shown in the figure below. The upper difference curve shows that the distinction between $E_i^1 E_i^1$ and $E_i^1 E_i^2$ would have been a little greater at 2 mmol/L fluoride concentration, but we elected to use 4 mmol/L to inhibit “usual” sera by about 80%. In the case of the much more frequent $E_i^1 E_i^1$, both concentrations of fluoride give the same distinction. The greater inhibition of the $E_i^1 E_i^1$ sera than the $E_i^2 E_i^1$, shown in the figure, was also observed by Garry (1). We, too, have observed the anomalous inhibition of atypical cholinesterase by fluoride when benzoxycholine is the substrate. This is another reason for the choice of substrate and inhibitor concentrations used in the Selected Method (2).

The data given in our figure do not entirely agree with those of King cited by McQueen, presumably taken from his reference 3. We cannot account for the differences; although the substrate concentration is not given, we doubt if it could account for the resulting differences.

References

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Expressing Lower Limits of Normal

To the Editor:

The problem of expressing lower limits of normal, addressed in the Letters of Altman and of Haymond and Knight [Clin. Chem. 25, 492–493 (1979)], lends itself to a practical solution, albeit a compromise. When the sensitivity of a test does not permit reliable measurements at concentrations near zero and (or) when there is no known clinical significance to low values (as with most enzymes), the “normal range” should be stated as “up to x,” where x is whatever has been established as an appropriate upper limit of normal. In these instances, a statement of the mean or median value may also be helpful. In addition, low concentrations may be reported as “less than y,” where y is the lowest concentration at which a reliable analytical result may be obtained. These approaches simplify both the analytical and interpretation problems attending low values.

We have used this system of reporting for some time and have received no objections from the medical community.

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Aperometric Liquid Chromatography of Catecholamines

To the Editor:

The work of Kissinger and co-workers (1, 2) offers a unique means for the electrochemical detection of catecholamines in biological fluids, because of the appropriate sensitivity of this detector, coupled with the resolving power of “high-performance” chromatography.

Used in actual biological measurements, this instrumental approach has at times been made confusing and unnecessarily difficult. We report here some modifications that should minimize difficulties with this methodology.

To separate norepinephrine and epinephrine (Figure 1) we use an Altex 110A pump and a 3.2 × 250 mm re-