CORRECTION

Nitrophenyl Acetate Hydrolysis by Acetylcholinesterase. A Correction

We wish to correct the values given in a previous paper for the rate of hydrolysis of *o*-nitrophenyl acetate by bovine erythrocyte acetylcholinesterase (Hastings *et al.*, 1970). The maximum hydrolysis rate for this substrate was reported to be much higher than that for the natural substrate, acetylcholine, and it was noted that this finding is of considerable importance because, according to mechanisms proposed earlier (Krupka, 1964, 1966b), the rate-limiting step in acetylcholine hydrolysis is deacetylation, a reaction common to the hydrolysis of all acetyl esters. Consequently no acetyl ester should be hydrolyzed significantly faster than acetylcholine. The mechanisms for substrate inhibition, for noncompetitive inhibition, and for the pH dependence of V_{max} with various acetyl ester substrates all depended on additions—of substrates, substrate analogs, or hydrogen ions, as the case may be—to the acetyl-enzyme intermediate, the latter occurring in a rate-limiting step in acetylcholine hydrolysis. All these mechanisms are therefore contradicted by the report, and even the postulate of an acetyl-enzyme intermediate becomes open to question, though independent evidence exists for this postulate as well.

On reexamination of the earlier paper we have noted that the numerical value of V_{max} for *o*-nitrophenyl acetate given in Table II, which is more than 50% higher than that for acetylcholine, is in disagreement with the experimental data shown in a Lineweaver-Burke plot in Figure 3 in the same paper. Recalculation of V_{max} from the latter data gives a figure close to that for acetylcholine (22.7 ± 0.8 compared to 19.4 ± 0.2 μmol min^{-1} ml^{-1}). The measurements have now been repeated in two different laboratories (first by R. M. K. and later by F. L. H.) and the rates for the two substrates found to be closely similar.

The conditions of the first experiment were as follows: pH 7.50, 26°, 0.1 *M* NaCl and 0.04 *M* $MgCl_2$, 2.0% methanol, with varying concentrations of *o*-nitrophenyl acetate. The total volume of the reaction mixture was 20 ml, and the initial rates of the reactions were determined by automatic titration of acid released with 0.01 *N* NaOH. The enzyme solution (bovine erythrocyte acetylcholinesterase from Sigma Chemical Co.) was standardized by determining rates of hydrolysis under the same conditions with 2 m*M* acetylcholine bromide. The maximum velocity for the latter was then calculated from the equation $V_{max} = v(1 + K_m/[S])$, where v is the measured velocity. K_m under these conditions had previously been found to be 3.20 ± 0.25 × 10^{-4} *M* (Krupka, 1966a). The pK_a value for *o*-nitrophenol is reported to be 7.23 at 25° (Albert and Serjeant, 1962). Accordingly, for each mole of *o*-nitrophenyl acetate hydrolyzed 1.65 mol of acid should be released at pH 7.5: *i.e.*, 1 mol from acetic acid and 0.65 mol from the partial ionization of *o*-nitrophenol.

The data were treated by means of a computer program giving a least-squares analysis for plots of $[S]/v$ against [S]. After applying standard statistical procedures and corrections for *o*-nitrophenol ionization, the desired parameters for the substrate *o*-nitrophenyl acetate were as follows: $V = 0.89 \pm 0.028$ relative to a V_{max} for acetylcholine of 1.00, and $K_m = 2.57 \pm 0.18 \times 10^{-4}$ *M*. The expectation that no acetyl ester substrate should be found with a significantly higher V_{max} than acetylcholine is therefore vindicated.

LITERATURE CITED

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