Letters to the Editor

Standardization of Clinical Enzyme Assays

We were disappointed by the first report of the joint DHSS/ACB working party on standardization of clinical chemistry enzyme assays which dealt with serum alkaline phosphatase (Moss, Baron, Walker, and Wilkinson, 1971). The principal message was that those who use a King-Armstrong method should standardize it by the manual method of King and Wootton (1956). Although most clinical chemists in the United Kingdom still use either a manual or mechanized King-Armstrong method, little help was offered to the increasing numbers of clinical chemists using either 6-glycerophosphate or p-nitrophenol phosphate as substrate. The relative merits of the three main substrate systems were regrettably not discussed and the problems associated with the use as secondary standards of commercially prepared dried sera containing added alkaline phosphatase from animal or avian viscera remain unanswered.

The stated aim of the working party is to recommend steps to improve the accuracy and reliability of diagnostic enzyme methods. We would state as general principles that (i) improved precision will be most readily achieved in the United Kingdom by the encouragement of mechanized methods in place of imprecise manual ones; (ii) the accuracy of a mechanized method depends upon it being closely correlated with the initial manual reference method. The reference method should be capable of a kinetic approach and carried out if possible under optimal conditions, e.g., Mg\(^2+\) concentration.

We conclude that methods using p-nitrophenol phosphate as substrate have certain advantages, such as better precision and kinetic capability over King-Armstrong methods. The modified Bessey-Lowry-Brock method can be easily adapted to ordinary AutoAnalyzer equipment as well as to the newer, more sophisticated analytical systems, and fortunately it appears that the liver and bone isoenzymes have similar affinities for p-nitrophenol phosphate as substrate.

The accuracy and precision of alkaline phosphatase determinations in the United Kingdom will not be significantly improved by the 'official' recommendation of a reference method which, although of great historical importance, still (1) will tacitly encourage the retention in smaller laboratories of existing inferior manual methods which may have a CV of 20 to 30%; (2) uses suboptimal conditions, e.g., Mg\(^2+\) concentration to measure arbitrary non-SI units; (3) causes difficulties when sera with high activity have to be assayed. Although the 'official' King-Armstrong method now has an incubation period of 0.25h it is suggested that the incubation period can be varied at will for high activity sera. Since it is not usually possible to alter easily the incubation time in mechanized systems, the working party are in effect recommending that most clinical chemists use a manual 'back-up' system for high activity sera.

We hope the working party will reconsider urgently their support for King-Armstrong methods and units.

A. D. HIRST
P. J. N. HOWORTH
Department of Chemical Pathology, King's College Hospital Medical School, London

References


The King-Armstrong Method

Some of the points raised by Hirst and Howorth (1972) in their criticism of our recommendations (Moss, Baron, Walker, and Wilkinson, 1971) on 'Standardization of alkaline phosphatase assays' have also been made to us by other biochemists and we, believe, based on a misapprehension of the purpose of our report.

We estimate on the basis of quality-control surveys that rather more than 60% of British clinical laboratories are carrying out alkaline phosphatase estimations by the King-Armstrong procedure or by its later modifications. The use of the AutoAnalyzer adaptation of this method is particularly widespread. Therefore, we feel that some agreement on the meaning of the King-Armstrong unit, and on the way in which automated procedures reporting results in this unit should be calibrated, would have a beneficial effect on the comparability of results reported by the majority of British laboratories. We appreciate that, as Hirst and Howorth suggest in their letter, kinetic methods with p-nitrophenyl phosphate as a substrate will probably increase in importance until the King-Armstrong unit finally lapses into disuse. However, an IFCC Expert Panel is currently engaged in attempting to define a reference kinetic method for alkaline phosphatase, and we did not wish to duplicate these efforts with the danger of reaching recommendations that might differ from those of the Expert Panel.

We are not able to confirm the estimations of a coefficient of variation of 20 to 30% (at an unstaed level of activity) quoted by Hirst and Howorth for the manual phenyl phosphate procedure. Recent estimates of this variation in our own laboratories have given figures of 10.1 ± 3.9% for the manual method and 9.2 ± 2.6% for the corresponding AutoAnalyzer procedure, at the 25 King-Armstrong units/100 ml level.

The results of any enzyme estimation could be expressed in SI units, if and when these have been agreed on as far as enzyme activity is concerned, but of course this will in no way affect the accuracy of precision of the methods. The difficulty in dealing with the high activity specimens inherent in all fixed-time methods and as Hirst and Howorth point out, is not worse in the AutoAnalyzer by the difficulty of altering the incubation period. A manual procedure for dealing with high-activity specimens might indeed be more preferable to dilution, with its risk of disproportionate changes in activity. However, it must be borne in mind that many automated kinetic procedures are equally inflexible with regard to such factors as their recording intervals and difficulties in interpretation of non-linear progress curves can arise in these methods also (Goldberg, Ellis, and Wilcock, 1971).

We hope that the comments of Hirst and Howorth will not deter those who are using the King-Armstrong method from giving careful consideration to our suggestions.

D. W. MOSS
D. N. BARON
P. G. WALKER
J. H. WILKINSON
Department of Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, London

References