Biochemical Pharmacology, Vol. 17, p. 1107. Pergamon Press. 1968. Printed in Great Britain

SHORT COMMUNICATIONS

Problems in the assessment of the drug-induced inhibition of enzymes

(*Received* 18 *December* 1967; *accepted* 31 *January* 1968)

A RECENT editorial article[1] discussed some problems encountered in the assessment of the drug-induced inhibition of enzyme activity, and drew attention to the difficulty of deriving a quantitative determination of the enzymic activity of crude tissue extracts. Enzyme assays using labelled substrates are often used in such studies, and one difficulty is that the presence of endogenous substrates in crude extracts can reduce the specific activity of the labelled substrate by an unknown amount. This can lead to a false inference of inhibition by drugs which alter the levels of endogenous substrates. The author recommended that enzyme samples should be subjected to some procedure, such as gel-filtration, which would remove endogenous substrates without altering enzymic activity.[2]

A cautionary article of this sort is most timely, but further comment seems necessary. It has been pointed out elsewhere that such purification by gel-filtration removes not only endogenous substrates, but also reversible inhibitors, and can, therefore, result in the erroneous conclusion that certain drugs are not inhibitors.[3]

Workers in this field ought to ask themselves what they are trying to measure, and also whether it is possible directly to measure such an effect. There are two possibilities. One might wish to determine either the actual *in vivo* level of enzymic activity in the presence of inhibitors and competing substrates, or the level of enzymic activity attainable in the absence of reversible inhibitors and competing substrates.

Disney[4] has recently drawn attention to the difficulty of measuring the activity of reversibly inhibited enzymes and pointed out that any dilution of the enzyme, or addition of substrate, during the assay reduces the inhibition and gives a false low level of inhibition. He concluded that no method of assay will give a true measure of the actual level of enzymic activity, and that the nearest approximation will be obtained by using an assay system which requires the minimum dilution of the enzyme sample, and the minimum addition of substrate. Radioactive methods of enzyme assay seem to be the only practicable methods to achieve the sensitivity, accuracy and specificity needed for such assays. Correction can be made for the presence of endogenous substrates by determining the specific activity of the labelled substrate in the sample by double isotope derivative analysis.[5]

In one wishes only to measure the activity, in the absence of competing substrates, of the enzyme which is not irreversibly inhibited, then simple techniques such as gel-filtration may conveniently be used to purify the crude extracts.

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