Evaluation of Cofactor Responsiveness

J. V. LEONARD and P. DAISH
Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK

The response to cofactor therapy in inborn errors of metabolism may be dramatic with complete resolution of the clinical illness but more commonly the response is absent or partial. When assessing cofactor responsiveness clinical and biochemical findings as well as the natural history of the disorder must be taken into account.

Several inborn errors of metabolism are characterised by defects in cofactor transport or metabolism. Much attention has been focused on those disorders in which the defect may be overcome by administration of the cofactor (or a precursor) in pharmacological doses. In some disorders (for example, biotinidase deficiency, congenital B12 malabsorption) the response to cofactor administration is dramatic with rapid and complete resolution of the clinical illness. However, in many conditions the response is less clear-cut; careful evaluation of clinical and biochemical data is then necessary to avoid erroneous classification of a non-responsive patient as responsive and vice versa. In this paper we will discuss some of the problems that may be encountered in the assessment of the response to cofactor therapy.

DEFINITION
A patient has a cofactor-responsive disorder if there is a substantial and sustained clinical and biochemical improvement following administration of the cofactor (or a precursor).

METHODS OF ASSESSMENT OF COFACTOR RESPONSIVENESS
We shall discuss the methods of assessment as follows:

Laboratory studies:
- enzyme activities and complementation groups
- metabolite concentrations
- response to loading tests.

Clinical measurements:
- growth and development
- degree of dietary restriction.

It should be emphasised that undue reliance on isolated biochemical data may be misleading. Similarly the natural history of the non-responsive disorder must be taken into account when assessing clinical data.

LABORATORY STUDIES

Enzyme and complementation studies
It is well recognised that the demonstration of enhanced enzyme activity following cofactor supplementation in vitro does not necessarily correlate with in vivo responsiveness (Kaye et al., 1974; Wilcken et al., 1977). This is found particularly with the cbl mutants of methylmalonic acidemia (McKusick 25110, 25111). Classification by complementation studies may have prognostic significance with regard to cofactor responsiveness. In a survey of patients with methylmalonic acidemia Matsui et al. (1983) found that out of 14 cbl A mutants, 13 showed significant falls in plasma and urinary methylmalonate with cofactor therapy whereas only four out of 11 cbl B mutants were cofactor-responsive.

METABOLITE CONCENTRATIONS
The measurement of changes in metabolite concentrations in body fluids provides an important means of assessing cofactor responsiveness. Evaluation of results is simple if the patient is metabolically in a steady state (or nearly so) before and during cofactor administration. Substantial or complete resolution of biochemical abnormalities following cofactor therapy suggests cofactor responsiveness. However, assessment under non-steady state conditions leads to difficulties. This is particularly true when cofactors are given to critically sick newly diagnosed patients; in these circumstances it may be impossible to separate the effects of cofactor therapy from those of other forms of treatment given at the same time. Figure 1 shows the progress of a baby girl with maple syrup urine disease (McKusick 28460) who was admitted at age 10 days. In addition to routine measures such as assisted ventilation, high carbohydrate intake and protein restriction she also received thiamine 60mg daily. Her plasma branched chain amino acid concentration fell rapidly and over the next few days the leucine intake (in the form of natural protein) necessary to sustain adequate plasma leucine concentrations rose to 200mg kg$^{-1}$ day$^{-1}$—the upper range for normal neonates (Francis, 1974). These findings could have been interpreted as a manifestation of cofactor responsiveness. However, her leucine requirements subsequently fell to low levels despite continued thiamine supplementation. At the age of 1 year (by which time thiamine had been discontinued) her leucine intake was 27mg kg$^{-1}$ day$^{-1}$. 
Leonard and Daish

Figure 1 Maple syrup urine disease and thiamine. There was a marked fall in plasma leucine concentrations concurrent with thiamine supplementation; however, tolerance of the high protein intake was not sustained once catch-up growth had been completed.

LOADING TESTS

Patients with milder or intermittent disease may have biochemical abnormalities only during illness. Thus, in order to demonstrate that cofactor therapy is of benefit, it may be necessary to induce biochemical abnormalities by stressing the patient. Loading tests before and during cofactor therapy are commonly employed for this purpose. For example, isoleucine may be given as a single oral load (100 mg kg\(^{-1}\)) to stress the metabolism of propionate. Meaningful comparisons between the responses to the isoleucine loads before and during cofactor therapy cannot be made unless several conditions have been satisfied. Of these the most important is that the patient be in a steady state prior to the loads and that these be carried out under near identical metabolic conditions. Results obtained otherwise will be misleading. Barnes et al. (1970) performed isoleucine loads on a male infant with propionic acidaemia. On the first occasion there was an appreciable and sustained rise in plasma propionate. After 5 days oral biotin therapy the isoleucine load was repeated. This time not only was the preload plasma propionate lower but also the rise in plasma propionate smaller and less prolonged. It was suggested from these data that he might be biotin-responsive. We have had the opportunity to review his subsequent progress. Biotin did not lead to increased tolerance of dietary protein nor was there a reduction in the frequency or severity of ketoacidemic episodes. He eventually died at the age of 4 years. It should be noted that prior to both loads he was "stabilised" on a diet containing only 0.1 g protein kg\(^{-1}\) day\(^{-1}\).

Our experience of single dose isoleucine loading tests indicates that reliable interpretation of results is extremely difficult. For this reason we suggest they be employed with great caution, if at all.

Longer term loading tests (over several days) are probably more reliable but also potentially dangerous since they may precipitate life-threatening ketoacidemic episodes in susceptible patients.

CLINICAL AND DIETETIC MEASUREMENTS

Long-term evaluation of cofactor responsiveness includes clinical and dietetic measurements. Cofactor therapy in a responsive patient will be associated with fewer and less severe metabolic crises, normal or improved growth and development, and tolerance of a normal or near-normal diet. However, when seeking evidence of cofactor responsiveness the natural history of the non-responsive disorder must be taken into account, as must the effects of other forms of therapy.

During periods of rapid growth in the neonatal period or early infancy a larger proportion of the protein intake will be used for growth leaving less to be catabolised (see Figure 1). A higher protein requirement also occurs during catch up growth later in infancy or early childhood. Patients with methylmalonic acidaemia commonly present at several months of age with failure to thrive. Once on a high energy–low protein diet they improve and during the period of increased growth may tolerate a relatively high natural protein intake of about 2–2.5 g kg\(^{-1}\) day\(^{-1}\) (Figure 2). If vitamin B\(_12\) be given during this growth spurt improvement may be falsely attributed to the effect of the vitamin.

Episodes of ketoacidemia in patients with organic acidaemias such as methylmalonic acidaemia and propionic acidaemia occur unpredictably. Despite the brittleness of their condition some patients will have prolonged periods of good metabolic control. The patient with methylmalonic acidaemia whose progress is shown in Figure 3 had numerous admissions with ketoacidemia during his first 2.7 years of life. After being placed in a special day nursery he had no further admissions for 10 months, an improvement that might