Inborn errors of metabolism

Vitamin-responsive genetic disease

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Vitamin-responsive genetic diseases are currently attracting a good deal of clinical and experimental attention and an effort will be made to review these with emphasis upon two questions: (1) from available evidence and theoretical considerations, what mechanisms underly known instances of vitamin responsiveness? (2) what implications does this knowledge have for improving therapy of these and related diseases?

No attempt will be made to cover the clinical features or to analyse in detail the biochemistry of the diseases discussed. Some of these aspects are covered in several recent reviews of vitamin-responsive (or 'vitamin-dependent') disease (Frimpter, Andelman, and George, 1969; Rosenberg, 1969, 1970; Mudd, 1971; Scrver, 1973) or can be found in the literature cited here. Some of these genetically determined conditions should perhaps more properly be termed 'traits' than 'diseases', since current evidence indicates the biochemical abnormality in at least some persons is not accompanied by clinical disease; for example, cystathioninuria due to cystathionase deficiency (Perry, Hardwick, Hansen, Love, and Israel, 1968b; Scott, Dassell, Clark, Chiang-Teng, and Swedberg, 1970).

In this paper a disease or trait will be considered to be responsive to a vitamin if some of its important and characteristic biochemical manifestations are alleviated by doses of a vitamin larger than that usually regarded as the normal requirement. This biochemical definition leaves open the question of whether the response is beneficial therapeutically. Table I lists those inherited diseases which at present have been shown to be vitamin-responsive. Thus at least 20 genetically determined diseases or biochemical abnormalities are now known to be responsive to one of seven vitamins and since knowledge is expanding rapidly, others may have been inadvertently overlooked. Certainly, there is reason to think that this list will be extended to include additional diseases and vitamins within the next few years.

Patients with a given enzyme deficiency are not uniform in their response to vitamin treatment; some patients respond while others do not. This is one manifestation of genetic heterogeneity and clarification of the basis for the vitamin responsiveness or non-responsiveness will lead to a greater understanding of the molecular variants in the disease. When the potential for vitamin response exists in a disease, each patient may merit a therapeutic trial even if other patients have failed to respond (Scrver, Mackenzie, Clow, and Delvin, 1971).

Mechanisms of Vitamin Responsiveness

Analysis of the mechanisms involved is favoured by that fact that in 14 of the 20 conditions listed in Table I (those indicated) the enzyme or protein abnormality has been established with reasonable certainty and in several of those remaining the presumed abnormality is localized to one of a few potential sites. In all cases the evidence is compatible with the vitamin response being due to an increased flow of metabolite through a reaction for which the vitamin, or one of its metabolites, is either a substrate or a cofactor. Flow through a particular enzyme reaction can be enhanced by two methods: (1) the conditions under which a fixed amount of enzyme is working are changed so that it functions at an increased rate, or (2) the amount of enzyme catalysing the reaction can be changed. Both methods are involved in the known examples of vitamin-responsive disease and will be illustrated by specific examples.

Defective metabolism of vitamins

The most straightforward situation is a genetic defect in the conversion of a vitamin to its metabolically active form (or forms). This may be a multistep process involving several protein factors and genetically determined abnormalities of these may lead to specific diseases. For example, in the case of cobalamin (vitamin B₁₂) several human
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Pyridoxine

*B12-Responsive convulsions probably due to glutamic acid decarboxylase deficiency (Frimpter et al, 1969; Yoshida et al, 1971).
*Cystathioninuria due to cystathionase deficiency (Mudd, 1971).
*Xanthurenic aciduria due to kynureninase deficiency (Tada et al, 1967; Tada et al, 1968).
*Oxaluria due to deficiency of soluble 2-oxo-glutarate-glyoxylate carboligase (Smith and Williams, 1967; Gibbs and Watts, 1970; Williams and Smith, 1972).

Vitamin B12

*Megaloablastic anaemia due to transcobalamin II deficiency (Hakami et al, 1971).

Folic acid

*Homocystinuria due to methylenetetrahydrofolate reductase deficiency (Freeman et al, 1972; Mudd et al, 1972; Shih et al, 1972).

Thiamine

*Maple syrup urine disease due to branched-chain keto acid decarboxylase deficiency (Scriber et al, 1971).
*Hyperpyruvic acidemia, hyperalaninemia, and hyperalaninuria due to pyruvate decarboxylase deficiency (Lonsdale et al, 1969; Blass et al, 1971).
*Protein-calorie malnutrition due to pyruvate dehydrogenase deficiency (Lonsdale et al, 1969).
*Lactic acidosis due to deficiency of low km pyruvate carboxylase (Brunette et al, 1972).

Lipoic acid


Biotin

*Propionic acidemia due to propionyl-CoA carboxylase deficiency (Barnes et al, 1970).
*β-Methylcrotonyl-CoA carboxylase deficiency due to β-methylcrotonyl-CoA carboxylase deficiency (Gompertz et al, 1971).

Vitamin D

Vitamin-D-dependent rickets
(a) X-Linked familial hypophosphataemic rickets (Williams and Winters, 1972; Glorieux et al, 1973).
(b) Pseudo-vitamin-D-deficiency rickets (Williams and Winters, 1972; DeLuca, 1973).

Table I Vitamin-responsive genetic diseases (1973)

Where review articles are cited these should be consulted to determine reporting priority and the sources of original contributions. Diseases in which the underlying protein abnormality is known are indicated by asterisks.

*Response to thiamine in reported cases has been equivocal.

Table II Genetic abnormalities in the handling of vitamin B12

The basis for literature citations is as in table I.

This abnormality is not known to have pathological consequences.

Two folic acid-responsive conditions can also be explained by the model illustrated in fig 1: congenital folate malabsorption and deficiency of methylenetetrahydrofolate reductase, an enzyme which converts one folate derivative to another. The pseudo-vitamin-D-deficiency form of vitamin-D-dependent rickets may well be another example of vitamin responsiveness brought about by an increase in the concentration of a substrate for a defective enzyme. Recent evidence indicates that this disease may represent a metabolic block in the conversion of 25-hydroxy-vitamin D3 to 1,25-dihydroxy-vitamin D3 (DeLuca, 1973).

It should be emphasized that although the simple model of a defective protein operating below saturation with its substrate can explain the response in all these abnormalities, there is no direct proof...
that this is in fact the operative mechanism. Such proof, when it is attempted, will require as a minimum an examination of the kinetic properties of the altered protein, establishment that under basal conditions substrate is available at a concentration below the Km, and demonstration that the concentration of available substrate is indeed raised by vitamin administration. It is important to realize also that this model may apply to mutant proteins for which either the Km or the maximal velocity, or both, have been altered (Mudd, 1971). In either case an increase in a non-saturating substrate may possibly achieve a sufficient flow through the reaction in question (fig 1).

In all these situations, the vitamin response takes advantage of the fact that lack of the product formed in the defective reaction, rather than toxicity due to substrate accumulation, accounts for the pathological consequences of improper metabolism of the vitamin. This fact, and the relative non-toxicity of most vitamins, set the conditions for benefit from further increases in substrate concentrations.

**DEFECTIVE BINDING OF VITAMIN COENZYMES TO SPECIFIC APOENZYMES**

At a time when there was little experimental evidence on the mechanisms of vitamin responsiveness, Scriver and Hutchinson (1963) pointed out that inherited modification of coenzyme binding by an apoenzyme might lead to a vitamin-responsive disease if the abnormal interaction could be overcome by relatively high concentrations of cofactor. This mechanism is logically appealing and has since gained widespread acceptance. However, in the opinion of the writer the operation of this mechanism in any specific disorder remains to be established. Experimental evidence suggesting the operation of this mechanism is limited to a very few cases in each of three vitamin B-responsive diseases. This experimental evidence will first be summarized, and then some of the remaining uncertainties will be discussed.

The results obtained by Tada and coworkers in studies of two unrelated children with vitamin B-responsive xanthurenic aciduria due to kynureninase (a pyridoxal-phosphate-dependent enzyme) deficiency are suggestive of the operation of this mechanism (Tada, Yokoyama, Nakagawa, Yoshida, and Arakawa, 1967; Tada, Yokoyama, Nakagawa, and Arakawa, 1968). These authors observed that reaction rates in normal human liver homogenates were increased by 20% by the addition of the cofactor. Kynureninase activity in liver homogenates of two children with B-responsive xanthurenic aciduria were quite low in the absence of added pyridoxal phosphate, but when high concentrations of pyridoxal phosphate were added, activity was restored almost to normal. This would certainly be expected if an abnormal interaction between cofactor and mutant enzyme had been overcome by increased cofactor.

Nevertheless, while such evidence makes the suggested mechanism plausible, it falls short of proving that this is actually at work, for several reasons. (1) Alternative, experimentally distinguishable explanations of these data are possible. For example, the mutant enzyme may be abnormally unstable in vitro and undergo rapid inactivation unless high concentrations of cofactor are present (Rosenberg, 1969). (2) No proof has been supplied that the cofactor concentration actually attained in vivo is high enough to support the required rate of catalytic activity. That an increase in vitamin B intake may not under some conditions be accompanied by an increase in tissue pyridoxal phosphate concentration is demonstrated by the recent work of Cohen et al (Cohen, Schneidman, Ginsberg-Fellner, Sturman, Knittle, and Gaull, 1973). These workers reported that an increase in the pyridoxine intake of rats from a dose equivalent to the high intake of 150-200 mg daily for a 70-kg man to a dose equivalent to the very high intake of 1500-2000 mg was accompanied by a decrease in pyridoxal phosphate concentration in liver and by no significant change in brain. (3) Before arriving at a final decision as to the prevalent mechanism of vitamin responsiveness in a given disease, it is most desirable to perform kinetic studies of the type performed by Tada and his coworkers on the enzymes from both responsive and non-responsive patients. It will be shown shortly...
that such studies in cases of cystathionase deficiency or cystathionine synthase deficiency may offer valuable and surprising insights into which kinetic properties measured in vitro are constantly associated with clinical responsiveness, and which are not, and may, therefore, be fortuitous.

Altered coenzyme binding has been implicated in cystathioninuria due to \( \gamma \)-cystathionase deficiency (fig 2). Enzyme assays have been reported for six patients with this relatively rare trait who were clinically responsive to vitamin B\(_6\) as shown by decreases in plasma and/or urinary cystathionine upon administration of high doses of pyridoxine (Frimpter, Haymovitz, and Horwith, 1963; Mongeau, Hilgartner, Worthen, and Frimpter, 1966; Berlow, 1966; Hooft, Carton, and Schryver, 1969). Crude liver extracts from three of these patients were assayed both with and without pyridoxal phosphate added in vitro. The liver extract from one of the patients studied by Frimpter (1965) showed a low \( \gamma \)-cystathionase activity which was restored almost to normal by the addition of pyridoxal phosphate (approximately 0.38 \( \mu \)mol added to an unspecified volume). The extract from a second patient had a low activity which was stimulated approximately four-fold by the addition of cofactor, but remained very low relative to control activities. The low activity in the extract from a third patient, studied by Finkelstein, Mudd, Irreverre, and Laster (1966), was stimulated only slightly by pyridoxal phosphate addition (final concentration \( 8 \times 10^{-4} \) mol/l), as was control \( \gamma \)-cystathionase activity, and attained a value of only 5\% of the mean control specific activity. The liver extracts from the three siblings studied by Hooft and coworkers (1969) were assayed only in the presence of \( 5 \times 10^{-5} \) mol pyridoxal phosphate/l. The \( \gamma \)-cystathionase activities were less than 6\% of the mean control value, whether or not the patients were receiving pyridoxine treatment at the time of assay.

Thus, of these six patients, in only one is the evidence compatible with the possibility that the sole abnormality is a binding defect for pyridoxal phosphate which is overcome by a high concentration of that coenzyme. The clinical responses of the other patients must have been based upon one or more other mechanisms. Indeed, in the absence of further evidence it is possible that even the first patient of Frimpter responded by virtue of such an alternative mechanism.

Evidence compatible with altered coenzyme binding has been reported for a single patient with a third condition: vitamin B\(_6\)-responsive convulsions and decreased renal activity of glutamic decarboxylase. Again, members of the group at Tohoku University found that a low enzyme activity measured in the absence of added cofactor could be restored to normal by in vitro addition of pyridoxal phosphate (Yoshida, Tada, and Arakawa, 1971).

It is of interest to note that the models which suggest that the mutant enzyme is unable to react normally with a cofactor are, in reality, very close to those discussed previously in which the mutant enzyme requires an unusually high substrate concentration to achieve a physiologically satisfactory rate of metabolite flow. In fact, these models may become identical in the case of a cofactor which rapidly equilibrates with the apoenzyme, since such a cofactor is kinetically indistinguishable from a substrate for the enzyme.\(^1\) In both instances, the beneficial therapeutic effect is attainable because

\(^1\) A means of analysing the interaction between an enzyme and a cofactor which associates with it relatively slowly and irreversibly has been described elsewhere (Mudd, 1971). This model may be applicable to most pyridoxal phosphate dependent enzymes. For example, decreased rates of formation of relatively irreversible complexes between mutant apoenzymes and pyridoxal-phosphate would explain the instances of in vitro stimulation of enzyme activities observed with kynureninase, cystathionase, and glutamic decarboxylase and discussed in this section.
high doses of vitamins and their derivatives can be given and tolerated.

**INCREASED STEADY-STATE CONCENTRATION OF ENZYME MUTANT WITH SLIGHT RESIDUAL ACTIVITY**

So far instances in which manipulation of the environment of a mutant enzyme results in an increased rate of metabolic flow through the step catalysed by that enzyme have been discussed. An alternative means of increasing such metabolic flow is to change the steady-state concentration of the enzyme itself. Present indications suggest that in most cases of vitamin B₆-responsive homocystinuria due to cystathionine synthase deficiency (fig 2), this is the operative mechanism. Only some patients with cystathionine synthase deficiency respond to vitamin B₆, and, within a sibship, all affected children are either responsive or not, suggesting that responsiveness has a genetic basis (Uhlendorf, Conerly, and Mudd, 1973). Several lines of evidence had already indicated that the vitamin B₆-response in this disease is due to an increase in the steady-state concentration of residual cystathionine synthase from a basal level of 1 to 2% of normal to the relatively modest level of 3 to 4% of normal. Previously published evidence is summarized as follows (Mudd, Edwards, Loeb, Brown, and Laster, 1970).

1 Changes in plasma and urinary amino acids during vitamin B₆ administration are characterized by decreases in homocysteine and its metabolites formed by enzymes proximal to cystathionine synthase, and by an increase in cysteine, the first metabolite measurable distal to the block.

2 No metabolites have been found during vitamin B₆ administration to suggest that an alternative pathway for homocysteine disposal has been stimulated.

3 In the few pyridoxine-responsive patients in whom assays of hepatic cystathionine synthase of sufficient sensitivity have been performed before and during vitamin B₆ administration, cystathionine synthase activity increased by three- to fourfold, but the activity during pyridoxine treatment is still only 3 to 4% of the mean control activity.

During the past several years, we have accumulated further results which support this hypothesis and which indicate the quantitative significance of a 1 to 2% residual activity of cystathionine synthase.

4 There is a striking correlation between clinical responsiveness to vitamin B₆ and the presence of residual cystathionine synthase activities in extracts of fibroblasts cultured from cystathionine-synthase-deficient patients. Table III summarizes our results on this, and updates the report of Uhlendorf et al (1973). Among responsive sibships, 16 had detectable enzyme activity, ranging from 10% to 0.1% of the mean control value. One exceptional responsive patient did not have significant cystathionine synthase activity in fibroblast extracts, but had previously been shown to have 1 to 2% residual activity in her liver (Mudd, Laster, Finkelstein, and Irreverre, 1967). Thus, all responders studied to date have some residual cystathionine synthase activity. On the other hand, among 10 non-responsive sibships, eight had no activity of cystathionine synthase detected by these sensitive assays. Two exceptional non-responders did have activity. Thus, residual cystathionine synthase appears to be a necessary, but not sufficient condition for clinical responsiveness. These results obtained with fibroblast extracts agree with measurements recently reported for liver extracts. Gaul and coworkers did not detect residual cystathionine synthase activities in liver extracts from two vitamin B₆-unresponsive patients 'before or after B₆'. Two responsive patients in their basal states had hepatic activities of 4% and 9% of normal (Gaul, Schaffner, and Sturman, 1973). The need for some residual activity of cystathionine synthase as a precondition of clinical B₆-responsiveness offers virtual proof that the B₆ response is mediated through cystathionine synthase and not by a stimulation of any other enzyme catalysing an alternative pathway for homocysteine disposal.

Studies of the effect in vitro of pyridoxal phosphate addition to the enzyme extracts from patients studied by Uhlendorf et al yielded interesting results. Generally speaking, the addition of pyridoxal phosphate stimulated the residual cystathionine synthase activities of responsive patients only to the same slight extent as occurred with normal enzyme. Surprisingly, a very striking stimulation—at least 10-fold—resulted from the addition of pyridoxal phosphate to the enzyme extract of one of the atypical patients who clinically did not respond to vitamin B₆ but whose fibroblasts had detectable enzyme activity. The latter finding serves as a warning that stimulation in vitro by a cofactor may not correlate with clinical responsiveness. By implication, when this effect in vitro is seen in a clinically responsive patient, there is still room for doubt whether the

| Number of Sibships (or Patients) with Cystathionine Synthase Activity* |
|-----------------------------|-----------------------------|
| Detected                  | Not Detected               |
| Responsive to B₆           | 16 (25)                    | 1 (1)                      |
| Not responsive            | 2 (2)                      | 8 (10)                     |

Table III Residual cystathionine synthase activity and vitamin B₆-responsiveness

*Unbracketed values indicate the number of sibships in each category; bracketed values, the number of individuals.
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stimulation is indeed the basis for the responsiveness.

5 If, as we have suggested, the activity of cystathionine synthase during pyridoxine response may be sufficient to metabolize most of the homocysteine arising from a normal dietary load of methionine, and this activity represents a two- to fourfold enhancement of the activity present in the basal state without vitamin B₆ treatment, then B₆-responsive patients in their basal states should be capable of metabolizing at least 25-50% of the normal homocysteine load. This would lead to the daily formation of at least 2-4 mmol of cystathionine, and hence, cysteine in such responsive patients (fig 2) and might be reflected by differences in the cystine requirements of vitamin B₆-responsive and non-responsive cystathionine-synthase-deficient patients.

To test this prediction we have measured the cystine required to maintain nitrogen balance in cystathionine-synthase-deficient patients in their basal states receiving normal amounts of vitamin B₆ (Poole, Mudd, Conerly, and Edwards, 1974). A typical non-responsive patient was in negative nitrogen balance (−0.96 g nitrogen/day) on a daily cystine intake of 155 mg, near the lower end of the 'zone of nitrogen equilibrium' (−0.38 g/day) on a cystine intake of 305 mg, and in nitrogen balance (+0.01 g/day) on a cystine intake of 505 mg. These results are comparable with those previously obtained by Brenton, Cusworth, Dent, and Jones (1966) with a B₆-non-responsive patient. This patient was in negative balance (approximately −1.12 g/day) on a cystine intake of 85 mg and in balance with an intake of 1085. Intermediate cystine intakes were not tested. From these data, a tentative estimate may be made that 300-500 mg of exogenous cystine is required daily for cystathionine-synthase-deficient patients who, being non-responders, are probably without residual activities of the deficient enzyme. The results obtained with vitamin B₆-responsive patients contrasts with this. None of the four B₆-responsive patients studied had a nitrogen balance significantly below the 'zone of nitrogen equilibrium', even on daily cystine intakes as low as 3-12 mg, the lowest intakes which could be achieved on the semi-synthetic diets used for these experiments. We conclude that these patients can form, endogenously from methionine, at least 300-500 mg daily or 2-5-4-2 mmol of cysteine. These amounts are roughly equivalent to 25-50% of the normal intake of methionine. Therefore, we regard these studies as strongly supporting the hypothesis that small residual activities of cystathionine synthase—1-2% of normal—may contribute metabolic capacities which are significant relative to the normal daily load. If such residual activities are enhanced (for example, by vitamin B₆ treatment) it then becomes reasonable to suppose that the normal load of homocysteine could be handled without undue accumulation of metabolites proximal to the block.

The mechanism whereby pyridoxine brings about this increase has not been established. We have shown that the cystathionine synthase of both control subjects and deficient patients is stabilized to heat inactivation by pyridoxal phosphate (Mudd et al, 1970). There is increasing evidence that inactivation of enzymes occurs chiefly in the apoenzyme form (see, for example, Litwack and Rosenfield, 1973, and literature cited there). Pyridoxine may then act by stabilizing mutant cystathionine synthase in vivo but other mechanisms are certainly possible.

Another possible example of a vitamin B₆ response due to a small increase in residual enzyme activity is suggested by a recent study of a single patient with primary pyridoxine-responsive sideroblastic anaemia and deficient δ-aminolevulinic acid synthetase activity (Aoki, Urata, and Takaku, 1973). The synthetase activity in extracts of erythroblasts of this patient, assayed in the presence of added pyridoxal phosphate, increased from 4% of the mean control value when the patient was in his basal state to 10% of the mean control value after one month's treatment with pyridoxal phosphate.

**Benefit of Small Increases in Already-Low Enzyme Activities**

These studies of vitamin B₆-responsive cystathionine synthase deficiency and δ-aminolevulinic acid synthetase deficiency demonstrate that it may be possible to produce a response by increasing the steady-state concentration of a mutant enzyme to a level which is still very low compared with the normal. That this situation may not be exceptional is shown by experience with variant forms of many genetic diseases. Thus table IV shows that patients with two different lipidoses, those with 12-44% of normal

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Proportion of Normal Activity (%)</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocerebrosidase</td>
<td>0-9</td>
<td>Classical infantile Gaucher's disease</td>
</tr>
<tr>
<td></td>
<td>12-44</td>
<td>Adult form of Gaucher's disease</td>
</tr>
<tr>
<td>Sphingomyelinase</td>
<td>0-7</td>
<td>Infantile form of Niemann-Pick disease</td>
</tr>
<tr>
<td></td>
<td>15-20</td>
<td>Adult form of Niemann-Pick disease</td>
</tr>
</tbody>
</table>

Table IV Enzyme activities and phenotypic expression in lipid storage diseases (adapted from Brady, 1973)
activity of the defective enzyme, are much less severely affected than those with 0-9%. In table V, residual activities of branched-chain keto acid decarboxylase are correlated with the clinical manifestations in variant forms of maple-syrup urine disease. An increase of this enzyme from 2% of normal to somewhat more than 8% transforms a severe life-threatening disease into a situation in which symptoms appear intermittently, if at all. Among the amino acidopathies, the presence of as little as 5 to 7% of normal phenylalanine hydroxylase activity prevents almost all the usual clinical difficulties associated with classical phenylketonuria (Kang, Kaufman, and Gerald, 1970; Friedman, Kaufman, and Kang, 1972). Finally, as shown in table VI, among patients with various lesions of hypoxanthine-guanine phosphoribosyltransferase, in most cases the possession of as little as 0-1% of normal activity is sufficient to prevent the most severe manifestations of the Lesch-Nyhan syndrome. However, the overlap in residual activities between patients with these differing clinical manifestations of hypoxanthine-guanine phosphoribosyltransferase deficiency emphasizes that the situation is really more complex than can be ascertained by a measurement of residual enzyme activity in a single cell type under one set of experimental conditions. Differences with respect to stability, kinetic properties, product inhibition, and so on may all play a role. As these differences are explored fully, relatively clear-cut distinctions such as those just made between cystathionine-synthase deficient-B6-responders and non-responders, and in tables IV-VI, can fade. A good example is afforded by studies of glucose-6-phosphate dehydrogenase variants (Kirkman, 1971). Nevertheless, it does appear that, as a general rule, a small amount of residual enzyme activity may be very beneficial. Conversely any therapeutic intervention which leads to this being enhanced, even to a slight extent only, may well be of great benefit.

**ENHANCEMENT OF ALTERNATIVE PATHWAYS**

In the instances considered so far, vitamin administration increases metabolite flow through a step which has been rendered inadequate because of a mutant protein. However, in several known instances of vitamin-responsive disease, it appears that the vitamin acts by stimulating flow through an alternative pathway catalyzed by a normal enzyme. Thus in lactic acidosis due to deficiency of the low Km pyruvate carboxylase, a decrease in accumulated lactate and alanine occurred after large doses of thiamine (Brunette, Delvin, Hazel, and Scriver, 1972). Thiamine pyrophosphate is not known to be a cofactor for the defective enzyme, pyruvate carboxylase, but is a cofactor for another enzyme which disposes of pyruvate, namely, pyruvate dehydrogenase (fig 3). Adding thiamine to the medium enhanced flow through the dehydrogenase step in assays with intact peripheral leucocytes from both control subjects and the patient with pyruvate carboxylase deficiency. Presumably a similar effect accounts for the clinical response to thiamine (Brunette et al, 1972).

The response to lipoic acid of some cases of a closely related disease, due to an almost complete

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**Table V**  Branched-chain keto acid decarboxylase activities and phenotypic expression (adapted from Zaleski et al, 1973)

<table>
<thead>
<tr>
<th>Proportion of Normal Activity (%)</th>
<th>Dietary Protein Tolerance</th>
<th>Diet Required</th>
<th>Clinical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>Inadequate to maintain nitrogen balance</td>
<td>Chiefly purified amino acids, low in branched-chain amino acids</td>
<td>Severe symptoms in first weeks of life</td>
</tr>
<tr>
<td>2-8</td>
<td>1-5-2 g/kg</td>
<td>Low protein</td>
<td>Symptoms often delayed and intermittent, induced by high protein intake or stress</td>
</tr>
<tr>
<td>&gt;8</td>
<td>Marginal or normal</td>
<td>Unrestricted</td>
<td>Possible acute symptoms during stress, especially infection</td>
</tr>
</tbody>
</table>

**Table VI**  Hypoxanthine-guanine phosphoribosyltransferase activities and phenotypic expression (adapted from Kelley and Arnold, 1973)

<table>
<thead>
<tr>
<th>Proportion of Normal Enzyme Activity (%)</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mostly &lt;0·1 (&lt;0·004 to 94)</td>
<td>Hyperuricaemia and hyperuric aciduria</td>
</tr>
<tr>
<td></td>
<td>Self mutilation</td>
</tr>
<tr>
<td></td>
<td>Choreaathetosis</td>
</tr>
<tr>
<td></td>
<td>Retarded mental and physical development</td>
</tr>
<tr>
<td>Mostly &gt;0·1 (0·01 to 32)</td>
<td>Hyperuricaemia and hyperuric aciduria</td>
</tr>
<tr>
<td></td>
<td>Gout</td>
</tr>
</tbody>
</table>
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Fig 3 A simplified diagram of the metabolic relationships pertinent to thiamine- or lipoic acid-responsive pyruvate carboxylase deficiency. Pyruvate carboxylase catalyses the conversion of pyruvate to oxaloacetate. Pyruvate decarboxylase (dehydrogenase) catalyses the conversion of pyruvate to acetyl-CoA.

absence of pyruvate carboxylase activity, may be analogous (Clayton, Dobbs, and Patrick, 1967; Hommes, Polman, and Rerrink, 1968). Another example is the response to pyridoxine of some cases of primary oxaluria due to deficient activity of soluble 2-oxo-glutarate-glyoxylate carboligase (Gibbs and Watts, 1970; Williams and Smith, 1972). In these cases it is thought that vitamin B₆ enhances the removal of accumulated glyoxylate by increasing flow through a pyridoxal-phosphate-dependent transaminase which catalyses the conversion of glyoxylate to glycine (fig 4). In none of these cases in which a normal enzyme activity is presumably enhanced by vitamin administration has the immediate mechanism by which the enzyme activity is affected been defined. One or more of the mechanisms discussed earlier in this paper may play a role.

The extent to which any of these manoeuvres is truly therapeutic must ultimately be evaluated in terms of clinical benefit to the patient. Situations in which a vitamin response is mediated through an effect on a normal, rather than a mutant, enzyme deserve particularly close scrutiny from this point of view, since in these situations intervention does not directly alleviate the primary abnormality, but introduces a second compensatory change from normal. In some instances the acute clinical benefit to the individual patient speaks for itself, as in the case of thiamine-responsive lactic acidosis reported by Brunette and colleagues (Brunette et al, 1972). Even in this case, the effect of attempts to restore metabolic flow through the primarily defective pyruvate carboxylase by treatment with biotin, the cofactor for this enzyme, remains to be evaluated (Brunette et al, 1972). In other, more chronic, diseases evaluation of the worth of a vitamin treatment may pose real difficulties. For example, the homocyst(e)ine accumulation which is the immediate result of cystathionine synthase deficiency may be decreased by treatment with folic acid (Carey, Fennelly, and Fitzgerald, 1968; Morrow and Barness, 1972), choline (Perry, Hansen, Love, Crawford, and Tischler, 1968a), or betaine (Komrower and Sardharwalla, 1971). During these treatments decreases in plasma and urinary homocyst(e)ine are usually accompanied by rises in methionine, as would be expected if remethylation of homocysteine had been enhanced (fig 2). Whether this alteration in the distribution of accumulated compounds is beneficial or not depends upon the extent to which homocyst(e)ine, on the one hand, or methionine on the other, accounts for the pathological consequences of cystathionine synthase deficiency. There are some indications that homocysteine is the chief toxic metabolite (McCully, 1970; McCully and Ragsdale, 1970; Kang and Trelstad, 1973), but the evidence is hardly adequate to permit more than a tentative judgment to be made.

Summary

The several ways in which vitamin administration may bring about a biochemical response in genetic abnormalities have been discussed. Two major interrelated lessons emerge from what we now know about vitamin-responsive genetic disease. First, it is possible to enhance metabolite flow through partially deficient reactions by suitable manipulation of the environment in which a fixed amount of enzyme functions or by changing the concentration of the enzyme itself. The latter approach may be the most versatile in the long run since there may be agents other than vitamins which increase enzyme concentrations. A striking example of such an effect in mammals is furnished by the work of Pirot and his collaborators, who by administration of casein hydrolysate to rats, increased threonine dehydratase activity several hundred-fold (Peraino and Pinot, 1964) by increasing the rate of enzyme synthesis (Jost, Khairallah, and Pirot, 1968). Other means of enhancing enzyme activities, ranging from tissue transplantation to transfer of genetic material, have been discussed elsewhere (for example, see Brady, 1973).
These procedures will not be discussed here, other than to mention a recent report (Mukherjee and Krasner, 1973) who transferred several small plugs of liver tissue (approximately 5% of the liver) from normal rats to the livers of rats genetically deficient in bilirubin uridinediphosphate glucuronotransferase activity. Twelve weeks later the specific activity of glucuronotransferase had risen in the livers of the recipient rats to 6-23% of normal, and the serum bilirubin of these rats, which had initially been elevated, had fallen to close to, or within, the normal range. Thus liver grafts between suitably matched individuals, may in the near future, become a means of increasing hepatic activities of deficient enzymes to extents which are therapeutically meaningful.

The second lesson to be learned from the review presented here is that enhancement of enzyme activity may be therapeutically beneficial even though the increase is small and the activity attained is still reduced relative to normal. It will be well to bear this in mind in any attempts to treat inborn errors of metabolism.

References


Vitamin-responsive genetic disease


