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The Biotin-Dependent Carboxylase Deficiencies

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INTRODUCTION

The carboxylase deficiencies have recently received much attention because of their increased recognition by geneticists and physicians, ease of laboratory diagnosis, and potential for treatment by protein restriction and vitamin supplementation. Disorders characterized by deficiencies of the four known human biotin-dependent enzymes have now been described (fig. 1). These enzymes are acetyl CoA carboxylase (ACC; E.C.6.4.1.2), a pivotal enzyme in the synthesis of fatty acids; pyruvate carboxylase (PC; E.C.6.4.1.1), which catalyzes the initial committed step in gluconeogenesis; propionyl CoA carboxylase (PCC; E.C.6.4.1.3), which catabolizes the branched-chain amino acids valine, isoleucine, methionine, and threonine, as well as the odd-chain fatty acids and the side chain of cholesterol; and β-methylcrotonyl CoA carboxylase (βMCC; E.C.6.4.1.4), which catalyzes the catabolism of leucine. In addition, a heterogeneous group of multiple carboxylase deficiencies have recently been characterized in which the activities of at least three mitochondrial biotin-dependent carboxylases are diminished. The purpose of this review article is to delineate and compare the clinical, biochemical, and genetic features of these inherited metabolic disorders.

Biotin and Biotin-Dependent Enzymes

Biotin was first recognized as an essential vitamin in living systems in 1936, when it was isolated as a yeast growth factor from egg yolk by Kögl and Tönnis [1]. The structure of biotin was determined by du Vigneaud et al. in 1942 [2, 3] and was first synthesized 2 years later by Harris et al. [4, 5]. Biotin ($C_{10}H_6O_3N_2S$) is a heterocyclic ring that is attached to an aliphatic side chain terminating in a carboxyl group (fig. 2). Of the eight different isomers, only d-biotin exhibits coenzyme activity and is found in nature. Small amounts of biotin are found in most

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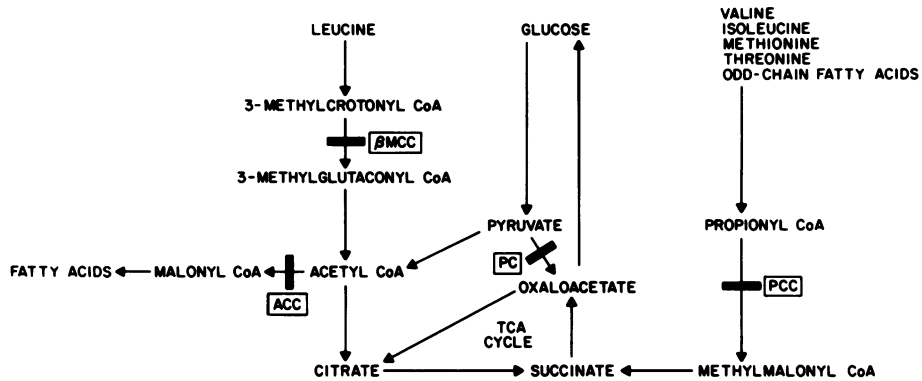

FIG. 1.—Metabolic pathways involved in the carboxylase deficiencies. Sites of PCC, PC βMCC, and ACC are indicated by *black bars*.

plant and animal tissues, and the vitamin is readily synthesized by a variety of bacteria.

Biotin serves as a covalently bound "CO_2" carrier for reactions in which a carboxyl group is fixed onto an acceptor (carboxylases), transferred from a donor to an acceptor (transcarboxylases), or liberated from a donor as carbon dioxide (decarboxylases). Of these, only the carboxylases are found in man. There have been several comprehensive reviews of biotin and of biotin-dependent enzymes [6–8].

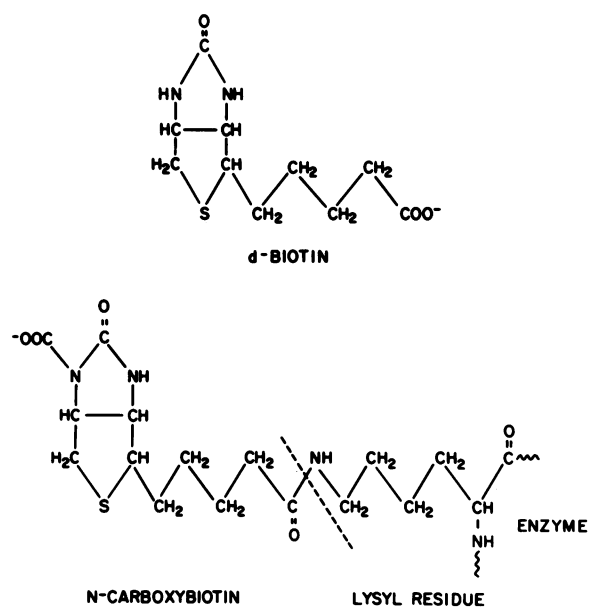

FIG. 2.—The structure of d-biotin and N-carboxybiotin covalently attached to ε-lysine residue of a carboxylase enzyme.

To function as a CO_2 carrier, biotin must be covalently bound through its carboxyl group to a lysyl ϵ-amino group on the apocarboxylase, thus forming active holoenzyme (fig. 2). Holoenzyme formation is catalyzed by one or more holocarboxylase synthetases [6, 9]. This reaction occurs as two partial reactions:

$$\text{(A)}\quad \text{d-biotin} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{d-biotinyl-5'-AMP} + PP_i$$

$$\text{(B)}\quad \underline{\text{d-biotinyl-5'-AMP} + \text{apocarboxylase} \rightarrow \text{holocarboxylase} + \text{AMP}}$$

$$\text{Net:}\quad \text{d-biotin} + \text{ATP} + \text{apocarboxylase} \overset{Mg^{2+}}{\rightarrow} \text{holocarboxylase} + \text{AMP} + PP_i$$

The formation of the biotinyl-5'-AMP intermediate (A) is followed by the transfer of the biotinyl moiety to the apoenzyme (B), forming active holoenzyme [10, 11]. The second partial reaction appears to be irreversible [12].

The action of biotin-dependent enzymes can also be expressed as two distinct partial reactions:

$$\text{(C)}\quad \text{Enzyme-biotin} + \text{ATP} + HCO_3^- \overset{Mg^{2+}}{\rightleftharpoons} \text{Enzyme-biotin-}CO_2 + \text{ADP} + P_i$$

$$\text{(D)}\quad \underline{\text{Enzyme-biotin-}CO_2 + \text{acceptor} \rightleftharpoons \text{Enzyme-biotin} + \text{acceptor-}CO_2}$$

$$\text{Net:}\quad HCO_3^- + \text{ATP} + \text{acceptor} \overset{Mg^{2+}}{\rightleftharpoons} \text{acceptor-}CO_2 + \text{ADP} + P_i$$

The initial reaction (C) involves the formation of the carboxybiotinyl enzyme using bicarbonate as the carboxyl donor. In the second reaction, (D), the carboxyl group is transferred from the enzyme-CO_2 complex to a specific acceptor substrate. This two-site "ping-pong" mechanism has been shown for all biotin enzymes studied.

Although two holocarboxylase synthetases, one located in the cytosol and specific for ACC and another located in the mitochondrion and specific for at least PCC, have been demonstrated in chicken liver [13, 14], similar studies have not been reported in mammalian systems. Holocarboxylase synthetases have not been found to be species specific [13], and the same synthetase may attach biotin to more than one carboxylase. None of the holocarboxylase synthetases have yet been completely purified, and their number, compartmentalization, and enzyme specificity remain to be determined.

Biochemical characteristics of the various human or mammalian carboxylases are presented in table 1. ACC is located in the cytosol, whereas the other carboxylases are mitochondrial [6]. The mitochondrial enzymes all have mol. wts. of about 500,000–540,000 daltons [15–17], but differ in their subunit compositions. Whereas PC is a tetramer consisting of four identical subunits, each about 130,000 daltons in mol. wt. [15], both PCC and βMCC are heterotetramers composed of four protomers, each consisting of two nonidentical subunits, α and β, with mol. wts. of 76,000–78,000 daltons and 52,000–54,000 daltons, respectively [16, 17]. The larger subunit, α, contains the biotin-binding site. PC is allosterically activated by acetyl CoA [6]; both PCC and βMCC are activated by monovalent cations

TABLE 1

BIOCHEMICAL PARAMETERS OF HUMAN AND MAMMALIAN BIOTIN-DEPENDENT CARBOXYLASES

Enzyme	Source	Mol. wt.	Subunit structure	Subunit mol. wt.	Activator	Reference
PC	Human	~500,000	α_4	~130,000	Acetyl CoA	[15]
PCC	Human	~540,000	$(\alpha\beta)_4$	72,000 56,000	Monovalent cations	[17]
βMCC.....	Bovine	~530,000	$(\alpha\beta)_4$	74,000 58,000	Monovalent cations	[16]
ACC.....	Rat	Polymeric	α_n	~250,000	Citrate	[19, 20, 22]

[6, 18]. ACC appears to be composed of large, 250,000 daltons, single polypeptides that polymerize in the presence of citrate [19–22].

Propionyl CoA Carboxylase Deficiency

Isolated deficiencies of each of the biotin-dependent carboxylases have been described. The most common of these, PCC deficiency or propionic acidemia, is the best characterized. Typically, affected children become symptomatic during the newborn period or in early infancy with vomiting, lethargy, and hypotonia [23]. Affected individuals accumulate propionate and its metabolites because of inadequate catabolism of isoleucine, valine, threonine, and methionine via the propionate pathway, which enters the tricarboxylic acid cycle through succinyl CoA. They excrete large concentrations of the organic acid salts, methylcitrate, 3-hydroxypropionate, and propionylglycine in their urine [24, 25]. Other minor organic acids that may be excreted in PCC-deficient patients, especially during episodes of metabolic decompensation, include tiglic acid and its glycine derivatives: 3-hydroxy-n-valerate, 3-oxo-n-valerate, 3-hydroxy-3-methylglutaric, and 2-methyl-3-hydroxybutyric acids [25, 26]. Excessive protein intake or intercurrent infections may precipitate episodes of organic acidemia, ketoacidosis, hyperglycinemia, and hyperammonemia [27, 28]. In addition, immunodeficiency has been described in at least one patient [29]. Recurrent episodes of metabolic decompensation caused by failure to diagnose or adequately treat the disease can eventually result in mental retardation, seizures, coma, and death. There, however, is considerable variation in the clinical expression of propionic acidemia in that some affected individuals manifest few or no symptoms, while others, even within the same family, may exhibit the classical features of the disease [30, 31]. The causes for this clinical variation are poorly understood. Patients with isolated PCC deficiency have been successfully treated by restriction of dietary protein [32], and in two documented cases, by the administration of oral biotin [33, 34].

Based on the results of a single newborn screening program, PCC deficiency is considered a rare disorder, with an estimated incidence of one in 350,000 individuals [35]. We have diagnosed or confirmed the diagnosis of about 25 new cases from the eastern and midwestern United States during the past 4 years, and we are cognizant of about 80 affected individuals. The pedigrees of these and other reported cases display no ethnic predilection and include both affected males and

females in simplex and multiplex sibships, several with a history of consanguinity [30], consistent with an autosomal recessive mode of inheritance.

Genetic heterogeneity in PCC deficiency has been established by genetic complementation studies in heterokaryons, generated by pairwise crosses of PCC-deficient fibroblast lines derived from unrelated patients. Various techniques have been used to assess complementation, including an autoradiographic method that detects [1−^{14}C]propionate utilization, measurement of propionate incorporation into trichloracetic acid precipitable material in intact heterokaryons [36], and direct determination of PCC activity in extracts of these heterokaryons [37]. These studies have demonstrated the existence of two major complementation groups among PCC-deficient lines, which have been designated *pcc A* and *pcc BC*. In addition to these major complementation groups, the *pcc BC* group can be further divided into three subgroups: *pcc B*, *pcc C*, and *pcc BC*. Of these, the *pcc B* lines complement all *pcc A* and *pcc C* mutants but not *pcc BC* mutants. Similarly, *pcc C* mutants complement all *pcc A* mutants and *pcc B* mutants, but not *pcc BC* mutants. Correspondingly, the *pcc BC* lines complement all *pcc A* lines, but neither *pcc B* nor *pcc C* lines. Based on these data and the known subunit composition of PCC, it has been proposed that the two major genetic complementation groups reflect defects in two different structural genes, probably corresponding to those coding for the α and β subunits of PCC [38–43]. In addition, the *pcc B*, *pcc C*, and *pcc BC* groups most likely represent allelic variants within the gene responsible for the major *pcc BC* complementation group. The variable clinical expression of PCC deficiency seems unrelated to complementation group, although, in general, individuals belonging to the *pcc A* group often fare worse than those of the *pcc BC* group. Clinical expression also varies among affected individuals within the same complementation group, implying that perhaps other genetic, environmental, or metabolic factors such as diet may influence the clinical expression.

PCC activities in extracts of cultured skin fibroblasts from both parents of the first reported case of propionic acidemia were approximately 50% of those observed in controls [44], suggesting that expression in obligate heterozygotes conforms to expected gene-dosage effects. However, in another report, PCC activity in peripheral blood leukocytes from both parents of an affected child was within the control range [45]. These apparent contradictory findings can be explained by the complementation group to which the individuals belong. When we measured PCC activity in extracts of cultured fibroblasts and peripheral blood leukocytes from 18 obligate heterozygotes for PCC deficiency, we found that the six heterozygotes from the *pcc A* complementation group had enzyme activities approximately half those of the controls, whereas all 12 heterozygotes from the *pcc BC* group had activities that were indistinguishable from those of controls [46]. Whereas the data from *pcc A* heterozygotes are consistent with the gene-dosage effects expected for simple additive interaction, those from *pcc BC* heterozygotes are not. It is likely that the *pcc BC* complementation group reflects mutations of the α subunit and the *pcc A* group mutations of the β subunit. If normal cells contain twice as many α as β subunits, either because α subunits are synthesized more rapidly or because they are degraded more slowly than are their counterparts, then a 50% reduction

in the number of normal α subunits, as would occur in cells from *pcc BC* heterozygotes, would balance the number of β subunits. This would conceivably permit a normal number of PCC protomers to form, thus yielding normal PCC activity. This hypothesis is predicated on the existence of a large pool of unused nascient α subunits in normal subjects. Regardless of the molecular mechanism, the PCC activities in tissues from obligate heterozygotes confirm the existence of two distinct mutant classes revealed by complementation analysis in cells from PCC-deficient probands.

PCC deficiency can be diagnosed prenatally either by demonstrating deficient propionate incorporation [47], deficient enzyme activity in cultured amniotic fluid cells [45], or by detecting the presence of elevated concentrations of methylcitrate in amniotic fluid [48]. In one reported case, PCC activity in amniotic fluid cells was normal but a significant amount of methylcitrate was detected in the fluid [49]. Subsequent analysis of fetal fibroblasts showed that the fetus was affected and the amniotic fluid cells were of maternal origin. Therefore, clearly both diagnostic procedures should be performed for accurate prenatal diagnosis.

Beta-methylcrotonyl CoA Carboxylase Deficiency

Beta-methylcrotonyl CoA carboxylase deficiency has been a suspected diagnosis in 10 published case reports [50–56]. Of these, seven were subsequently found to have multiple carboxylase deficiency [50, 52–54, 56]. Isolated βMCC deficiency was enzymatically confirmed in only one, an Indian female of nonconsanguineous parents. A mitochondrial preparation from her liver had no detectable βMCC activity, but did have normal PCC activity, excluding the diagnosis of multiple carboxylase deficiency [55]. At 11 weeks of age, the proband exhibited difficulty feeding, tachypnea, irritability, lethargy, and infantile spasms. She had a mild metabolic acidosis with slightly elevated serum pyruvate and lactate concentrations. Her urine contained ketones, β-hydroxyisovaleric acid, and β-oxoglutaric acid, but no detectable β-methylcrotonylglycine. The patient died at 13 weeks of age before the effects of biotin treatment could be evaluated.

A second patient, a 4½-month-old female of consanguineous parents presented at 2 weeks of age with feeding difficulties and hypotonia [51]. Her deep tendon reflexes were absent, and she had tongue fibrillations resembling those in Werdnig-Hoffman disease, but she was never ketoacidotic. Her urine had the odor of cat's urine and contained large amounts of β-hydroxyisovaleric acid and β-methylcrotonylglycine. The patient's clinical course did not significantly improve on a low-leucine diet supplemented daily with 0.25 mg of biotin; she died of pneumonia at 9 months of age. No enzymatic studies were performed on her tissues.

The third patient, also a female and the second child of consanguineous Pakistani parents, first presented at 1 month of age with spasms that progressed to generalized seizures and elevated urinary β-hydroxyisovaleric acid and lactate [56]. She improved following the administration of 5 mg biotin daily, but exhibited some residual neurological damage. Tissues were not available for enzymatic analysis.

Only the first patient described above can be considered to unequivocally have had isolated βMCC deficiency. The third patient may have represented another case of multiple carboxylase deficiency. Further characterization of isolated βMCC deficiency, therefore, must await the careful evaluation of additional patients. Such an evaluation should include diagnostic confirmation by enzymatic analysis and subsequent clinical assessment of biotin-responsiveness.

Pyruvate Carboxylase Deficiency

Because of the pivotal role of PC in the regulation of the tricarboxylic acid cycle as well as providing intermediates in numerous other pathways, individuals with PC deficiency exhibit a variety of clinical and biochemical abnormalities. PC deficiency has been suggested as a possible cause of Leigh's subacute necrotizing encephalomyelopathy (SNE), a degenerative disorder of the brain stem frequently characterized by biochemical features similar to those described for PC deficiency. However, recent studies [57] indicated that SNE and PC deficiency are probably distinct entities, and a review of patients with SNE who were found to have undetectable PC activity in their tissues suggests that perhaps the tissues studied were preserved and/or measured under less than optimal conditions, leading to falsely deficient PC activities.

Twenty-one cases of probable PC deficiency have been reported [58–75], although inadequate storage or assay conditions have confounded the diagnosis in three of these patients [61, 63, 66]. The most common clinical findings are metabolic acidosis, hypotonia, markedly delayed physical and mental development, and seizures. The onset of symptoms generally first appears in early infancy, and the majority of patients expire shortly thereafter. Those who have survived are severely retarded [75]. Serum lactate concentrations, usually accompanied by increased pyruvate and alanine concentrations, have been elevated in all reported cases of PC deficiency. Despite the absence of PC activity, hypoglycemia has not been a consistent finding in this disorder. In addition, because of elevated acetyl CoA concentrations (via the pyruvate dehydrogenase pathway), fatty acid synthesis, sterol, and ketone body synthesis may also be elevated, thus emphasizing the central role of PC in the regulation of lipid metabolism.

Treatment of PC deficiency has focused mainly on replacement of the missing four-carbon tricarboxylic acid cycle intermediates, in most cases without substantial clinical improvement [76]. Administration of pharmacological doses of biotin has not been successful. Some clinical improvement with thiamine treatment, conceivably by shunting pyruvate through the pyruvate dehydrogenase pathway, has been reported in one patient [58].

Like PCC deficiency, PC deficiency appears to be inherited as an autosomal recessive disorder. DeVivo et al. [71] reported PC activities in the fibroblasts of the parents of an affected patient that were intermediate between those of the deficient patient and the controls. Other investigators have shown similar intermediate PC activities in obligate heterozygotes [72–74, 77]. Nine males and nine females and three of unspecified sex have been reported, including three families in which more

than one sibling has been affected [67, 74, 75]. Consanguinity has been reported in at least one family [73].

Prenatal diagnosis of PC deficiency, a possibility suggested by Hansen and Christensen [78] and Feldman and Wolf [79], has been successful by demonstrating deficient PC activity in the amniotic fluid cells [80]. The results were corroborated by four independent laboratories, and the diagnosis was confirmed by the absence of measurable PC activity in all examined tissues of the aborted fetus.

Genetic heterogeneity in PC deficiency has also been investigated by comparing the enzyme activity in polyethylene glycol-induced heterokaryons with that in unfused mixtures of fibroblasts from affected children ([81] and B. Wolf, unpublished results). Complementation was not observed among the cells of four patients with isolated PC deficiency (*pyc* complementation group), but was observed in fusions between each of these lines and a biotin-responsive multiple carboxylase-deficient fibroblast line (*bio* complementation group; see *Multiple Carboxylase Deficiency* below). Kinetic analysis of complementing PC-deficient lines revealed that maximum restoration was achieved within 10–24 hrs after fusion, a profile similar to that achieved between the *bio* line and lines belonging to the *pcc* complementation groups, known to represent different gene mutations. These studies suggest that the mutations in all *pyc* cell lines examined are likely interallelic. This result is expected if human PC is a tetramer composed of identical subunits [15].

Acetyl CoA Carboxylase Deficiency

A recent preliminary report described the first recognized case of isolated ACC deficiency [82]. The patient, a newborn female with hypotonic myopathy and neurological damage, excreted urinary metabolites of hexanoic acid, including 2-ethyl-3-keto-hexanoic acid, 2-ethyl-3-hydroxy-hexanoic acid, and 2-ethyl-hexanedioic acid. The diagnosis was confirmed by finding deficient ACC activity in her liver compared to that in rat liver and about 10% of normal ACC activity in her fibroblasts.

Multiple Carboxylase Deficiency

In 1971, Gompertz et al. described a newborn with metabolic ketoacidosis, β-methylcrotonylglycinuria, and β-hydroxyisovaleric aciduria. Following biotin treatment, he improved clinically and no longer excreted abnormal urinary metabolites [50]. In addition to confirming βMCC deficiency in his fibroblasts [83], PCC deficiency was demonstrated [84, 85]. Several other patients with biotin-responsive β-methylcrotonyl CoA carboxylase deficiency [52–54, 56] have subsequently been shown to have deficient activities of PCC [83–89] and PC [86, 89]. Additional patients with multiple carboxylase deficiency have been described [90–98].

Based on the age of initial clinical presentation as well as on biochemical data, two distinct types of multiple carboxylase deficiency can be discriminated: a neonatal or infantile form and a late-onset or juvenile form (table 2) [99]. The neonatal form is usually manifested in the first few days of life by vomiting, lethargy, and hypotonia, associated with metabolic ketoacidosis, lactic acidosis, and eleva-

TABLE 2

TWO FORMS OF MULTIPLE CARBOXYLASE DEFICIENCY

	Neonatal form	Late-onset form (~3 mos)
Clinical features	Vomiting, lethargy, hypotonia	Skin rash, conjunctivitis alopecia, candidiasis, ataxia developmental delay
Biochemical features	Keto-lactic acidosis, organic acidemia, hyperammonemia	Keto-lactic acidosis, organic acidemia
Serum biotin levels	Normal	Low
Urinary biotin levels	Normal	Low
Leukocyte carboxylase activities:		
Before biotin	Deficient	Deficient
After biotin	Near-normal or normal	Normal
Fibroblast carboxylase activities:		
Before biotin	Deficient	Normal
After biotin	Near-normal or normal	Normal
Defect	Holocarboxylase synthetase	Biotin absorption or transport?

tions of the various abnormal urinary metabolites characteristic of the isolated carboxylase deficiencies [91, 92]. Whenever measured, carboxylase activities were deficient in the patients' peripheral blood leukocytes and their serum biotin concentrations were normal. Most patients have improved clinically after treatment with pharmacologic doses of oral biotin, and, when examined, the activities of their leukocyte enzymes have approached normal. Furthermore, deficient carboxylase activities in cultured skin fibroblasts from these patients increased to normal or above normal when incubated in high concentrations of biotin. Several investigators have recently presented evidence that the primary enzyme defect is in holocarboxylase synthetase, the enzyme that covalently attaches biotin to the various apocarboxylases [100, 101]. The K_m of biotin for the synthetase in two of these *bio* lines was about 350 times that for the synthetase in normal cells. Affected males and females within the same sibships support an autosomal recessive mode of inheritance for this disorder.

Positive genetic complementation has been found in all fusions of the neonatal form of multiple carboxylase-deficient fibroblast lines, (*bio*), with either *pcc* or *pyc* cell lines when compared to unfused cell mixtures [87, 102, 103]. All *bio* lines have thus far failed to complement each other with respect to the various carboxylase activities. Genetic heterogeneity within the multiple carboxylase-deficient lines may be revealed by genetic complementation experiments when holocarboxylase synthetase activity can be assayed directly.

Biochemical heterogeneity within the *bio* group has been demonstrated by comparing the carboxylase activities of two multiple carboxylase lines in response to biotin [104]. The carboxylase activities in most *bio* lines are stimulated to normal or above-normal within 24 hrs of incubation with as little as 0.1 to 1 mg/l biotin. However, we have investigated another *bio* line in which 4–6 days were required for the carboxylase activities to reach maximum activities of 30%–60% of normal even when incubated with as much as 10 mg/l biotin. The differences between these two *bio* lines may reflect differences in the K_m of biotin for their respective

altered holocarboxylase synthetases. One of the cases reported by Bartlett et al. [89, case 4] may prove to have properties similar to our *bio* variant line.

ACC activity has been shown to be deficient in at least two *bio* lines [105, 106]. Since ACC is a cytosolic enzyme, whereas the other carboxylases are mitochondrial, deficient activities of all four biotin-dependent enzymes suggests that biotin attachment may occur by several possible alternative mechanisms. These include a common holocarboxylase synthetase attaching the biotin to enzyme subunits or assembled apoenzymes prior to transport into the mitochondria, identical synthetases in both compartments, or synthetases with common subunits.

Neonatal multiple carboxylase deficiency has been successfully diagnosed prenatally [107]. Both this case and another undiagnosed fetus at risk for multiple carboxylase deficiency have been treated prenatally with pharmacological doses of biotin [107, 108]. The latter child was subsequently shown to be affected when postnatal withdrawal of biotin supplementation resulted in metabolic decompensation. Both children have responded well to continuous biotin therapy. Whether it is necessary to begin biotin treatment of an affected fetus prenatally or immediately after birth remains to be determined.

Eight cases of the late-onset form of biotin-responsive multiple carboxylase deficiency have been reported [93–98]. These patients have clinical features similar to those of patients with the neonatal form of multiple carboxylase deficiency (*bio* complementation group) with deficient leukocyte carboxylase activities before biotin therapy that increase to normal after biotin treatment. However, in contrast to the neonatal form, the onset of the disease in these infants is delayed until the child is 3–6 months of age and the carboxylase activities in fibroblast extracts are normal regardless of the biotin concentration in the culture medium. In addition, the serum biotin concentrations, at one-quarter to one-third of the mean normal values, overlap with the low normal range, whereas the urinary biotin concentrations are well below normal. When varying concentrations of biotin were administered orally to a patient with the late-onset form of multiple carboxylase deficiency, his plasma biotin concentrations did not increase to the concentrations evoked in similarly tested controls [108]. This finding has led to speculation that the probable defect in the late-onset form involves an abnormality in the intestinal absorption of biotin [109].

Munnich et al. [110] described a 1-year-old child with features of multiple carboxylase deficiency, including total alopecia, erythematous skin eruptions, and kerato-conjunctivitis. In addition to demonstrating deficiencies of the mitochondrial carboxylases, these authors presented clinical evidence of ACC deficiency, including decreased concentrations of linoleic acid. When the child was treated with oral and cutaneous unsaturated fatty acids a dramatic improvement of his skin lesions resulted. This response was independent of biotin therapy, whereas the other biochemical abnormalities were corrected only after biotin therapy was begun. However, no measurement of ACC activity in any tissues was presented.

Immunological dysfunction has been described in two patients [111, 112], one demonstrating defects in both T-cell and B-cell immunity [111] and the other described above with probable ACC deficiency [110], having a defect in the im-

munoregulatory system [112]. The findings in the latter case could be explained by a reduction in the synthesis of prostaglandin E_2, a major product of ACC-dependent fatty acid metabolism and an activator of the in vitro immunoregulatory system.

Care must be taken to differentiate the inherited multiple carboxylase deficiencies from acquired biotin deficiencies, such as from excessive dietary intake of avidin, an egg-white glycoprotein that binds specifically and essentially irreversibly to biotin [113], or by prolonged parenteral alimentation without supplemental biotin [114, 115]. Patients with an acquired biotin deficiency can be maintained on physiological doses of biotin, whereas those with inherited multiple carboxylase deficiencies always require pharmacological doses of biotin to avert metabolic imbalances.

CONCLUSION

The metabolic disorders caused by individual deficiencies of each of the four carboxylases as well as by the two forms of multiple carboxylase deficiency have been described. PCC, PC, and multiple carboxylase deficiencies are well-characterized clinical diseases, whereas the delineations of both isolated βMCC and isolated ACC deficiency await subsequent case studies. Although individuals with the same enzyme deficiency may have variable clinical features, generally all of the carboxylase deficiencies are manifested in neonates, infants, or young children by feeding difficulties, hypotonia, and lethargy associated with metabolic acidosis and excess serum and urine concentrations of the respective organic acids. Treatment usually involves restriction of dietary protein or, specifically, the amino acid or acids not effectively catabolized. Most cases of multiple carboxylase deficiency, whether of the neonatal or late-onset form, have improved clinically upon treatment with pharmacological doses of biotin. Only two individuals with isolated PCC deficiency and no patients with isolated PC deficiency have been biotin-responsive. Since biotin is covalently attached to the apoenzymes, it seems unlikely that an individual with an isolated carboxylase deficiency caused by a structurally abnormal enzyme would respond to biotin treatment. On the other hand, biotin therapy may be essentially curative in the patient with multiple carboxylase deficiency. The success of dietary treatment, biotin therapy, or both depends upon the extent of irreversible damage sustained before the therapy began.

The association of immune dysfunction with carboxylase deficiency is of great clinical importance. To date, such dysfunctions have been described in isolated PCC deficiency and the late-onset form of multiple carboxylase deficiency. Whether the immune defects are caused by a failure to synthesize appropriate enzyme products, by the accumulation of abnormal metabolites, or simply by malnutrition in compromised patients awaits further study.

The structures of human PCC and PC have been biochemically determined and are similar to those of lower mammals. The various carboxylases also resemble each other in molecular weight, biotin content, and overall enzymatic reaction mechanism. The subunit compositions differ, however, possibly because of evolutionary divergence. Despite preliminary evidence suggesting that PCC was a tet-

ramer of identical subunits, genetic complementation studies of PCC-deficient fibroblast lines predicted that it was composed of nonidentical subunits. Subsequent structural studies of the purified enzyme confirmed this subunit composition. Positive complementation has not yet been demonstrated among isolated PC-deficient fibroblast lines, consistent with the identical subunit structure of the human enzyme.

The occurrence of PCC, PC, and multiple carboxylase deficiencies in males and females, with consanguinity reported in several of these families, is consistent with an autosomal recessive mode of inheritance for these disorders. This conclusion is further supported by the demonstration of intermediate enzyme activities in obligate heterozygotes of PC deficiency and for some carriers of PCC deficiency. Although carboxylase activities are normal in obligate heterozygotes belonging to the *bio* group, intermediate holocarboxylase synthetase activity is expected in cells of these carriers once this enzyme is assayed.

PCC deficiency, PC deficiency, and the neonatal form of multiple carboxylase deficiency have been diagnosed prenatally by direct measurement of the relevant carboxylase activity in cultured amniotic fluid cells. In addition, PCC deficiency has been prenatally detected by the presence of methylcitrate in the amniotic fluid. Prenatal diagnosis of βMCC deficiency may also be possible by measuring β-hydroxyisovaleric acid in the amniotic fluid of an affected fetus.

Studies of the biotin-dependent enzymes and their deficiency disorders have provided much information not only on a group of related, treatable inherited metabolic diseases, but also on the contribution of the carboxylases to basic intermediary metabolism. Nonetheless, the clinical and biochemical delineation of βMCC and ACC deficiencies, the intracellular compartmentation and biosynthesis of the various carboxylases, the mechanism of immune dysfunction in the carboxylase deficiencies, the relationship of biotin metabolism to enzyme function and possible treatment, and the elucidation of secondary effects of the abnormal metabolites on various other biochemical reactions remain important, but unresolved, problems for future investigation.

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