Ann. Rev. Nutr. 1986. 6:317–43

INHERITABLE BIOTIN-TREATABLE DISORDERS AND ASSOCIATED PHENOMENA

Lawrence Sweetman and William L. Nyhan

Department of Pediatrics, University of California, San Diego, La Jolla, California 92093

CONTENTS

INTRODUCTION

Although the vitamin biotin was discovered some sixty years ago, there was until recently relatively little emphasis on the study of biotin in man. This was because of the extremely rare occurrence of human clinical deficiency of biotin and the lack of knowledge of the associated biochemical abnormalities. The rarity of biotin deficiency is a consequence of the small daily requirement for this water-soluble vitamin, the broad distribution of biotin in foods, and the synthesis of biotin by intestinal flora. The recent increased interest in the metabolism of biotin was stimulated by the discovery in the last fifteen years of infants and children with inherited defects in the metabolism of biotin and by

0199-9885/86/0715-0317$02.00

the fact that they can be successfully treated with pharmacological doses of biotin. Two forms of "biotin-responsive multiple carboxylase deficiency" have greatly increased our knowledge about the clinical and biochemical consequences of abnormalities of biotin metabolism and of the deficiency of biotin. An excellent symposium on biotin was published recently (38).

History of Biotin

In the 1920s and 1930s it was discovered that a diet high in raw egg white caused dermatitis, hair loss (alopecia), and neurologic abnormalities in rats (23, 24, 117, 118). These could be prevented by a "protective factor X" present in potato starch, yeast, egg yolk, and milk (24) as well as liver (117). A similar dermatitis in chicks fed a diet rich in egg white was corrected by liver extract (91). A factor protective against egg white injury was found in liver and yeast and named vitamin H (62, 63). A yeast growth factor initially isolated from yeast and then egg yolk was named biotin (83) and later noted to have vitamin H activity (44, 66). The structure of biotin was determined in 1942 (43, 97) and vitamin H and biotin were shown to be identical in microbiological assays (89). A legume nodule respiration factor, coenzyme R, was also shown to be identical to biotin (159). Biotin was first synthesized in 1943 (69), the crystal structure determined in 1956 (155), and the stereospecific total synthesis of the natural isomer *d*-(+)-biotin was accomplished in 1975 (35, 109).

The structure of the natural isomer, *d*-biotin, shown in Figure 1, contains a ureido group in a five-membered ring fused with a tetrahydrothiophene ring with a five-carbon side chain terminating in a carboxyl group. Biotin is synthesized biochemically from pimelic acid, a seven-carbon dicarboxylic acid, L-alanine, and L-cysteine by a variety of microorganisms (75). The biochemistry and genetics of the synthesis of biotin have been particularly well studied in *E. coli* (46).

The agent in raw egg white causing the toxic manifestations that are reversed or prevented by biotin is the protein avidin (45). Avidin binds biotin with a very

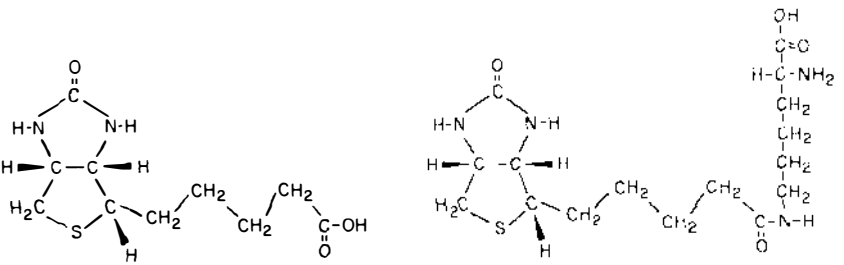

Figure 1 Structures of biotin and biocytin.

high affinity, the dissociation constant is 10^{-15} molar (60). Thus ingestion of large amounts of avidin leads to the formation of biotin-avidin complexes and prevents the absorption of biotin; this creates a deficiency of biotin and the resulting symptoms (64, 65).

Metabolic Role of Biotin and its Metabolism

In mammals and birds, biotin is a covalently bound cofactor in four enzymes, all carboxylases involved in the fixation of carbon dioxide and requiring adenosine triphosphate (ATP) (102). Acetyl-CoA carboxylase catalyzes the formation of malonyl-CoA from acetyl-CoA, bicarbonate, and ATP. Malonyl-CoA is then utilized in fatty acid synthesis and fatty acid chain elongation. Thus biotin is essential for lipogenesis.

Pyruvate carboxylase catalyzes the synthesis of the tricarboxylic acid cycle intermediate, oxaloacetic acid from pyruvate, bicarbonate, and ATP. This provides an intermediate to prime the cycle as well as providing a source of carbon skeletons for the amino acids aspartate and glutamate derived from the cycle. In the gluconeogenic tissues liver and kidney, the oxaloacetic acid is utilized for the synthesis of glucose. Thus biotin plays a critical role in energy metabolism and in synthesis of amino acids and glucose.

Propionyl-CoA carboxylase catalyzes the carboxylation of propionyl-CoA to methylmalonyl-CoA, which is then isomerized to succinyl-CoA and enters the tricarboxylic acid cycle. Thus biotin is essential for the catabolism of propionic acid, which is derived from intestinal flora, from the catabolism of the amino acids isoleucine, valine, methionine, and threonine, the side chain of cholesterol, and from the oxidation of odd-numbered fatty acids.

3-Methylcrotonyl-CoA carboxylase forms 3-methylglutaconyl-CoA from 3-methylcrotonyl-CoA in the catabolic path of the amino acid leucine. Acetyl-CoA carboxylase is localized in the cytosol: the other three carboxylases are localized in the mitochondria. The function of biotin in the carboxylases is to carry the carbon dioxide. Carboxybiotin is formed with energy provided by ATP and the carboxyl group transferred to the organic acid substrate.

The carboxylases are synthesized as enzymatically inactive apocarboxylases lacking biotin. Enzymatically active holocarboxylases are formed by the covalent attachment of biotin to the apocarboxylases, catalyzed by the enzyme holocarboxylase synthetase (1). Holocarboxylase synthetase catalyzes two sequential reactions. The first is the activation of biotin with ATP to form biotinyl-adenylate. This then is reacted with the epsilon amino group of a lysine in the active site of the apocarboxylase, forming a covalent amide bond of *d*(+)biotinyl-ε-*N*-L-lysine. This biotinylated lysine is named biocytin.

Holocarboxylase synthetase is present in both the cytosol and mitochondria but it is not yet known whether biotin is attached to the mitochondrial carboxylases before or after their incorporation into the mitochondria. As discussed

below, the properties of human mutant holocarboxylase synthetase indicate that both the mitochondrial and cytoplasmic forms are encoded by one gene and that the same holocarboxylase synthetase attaches biotin to all four apocarboxylases. Holocarboxylase synthetase from species as disparate as rabbit, yeast, and bacteria can attach biotin to apocarboxylases from the different species (95). In all of the carboxylases for which the amino acid sequences around the biocytin are known, the lysine is flanked by methione residues (174).

In the normal turnover of the holocarboxylases in cells, they are degraded by proteolysis to small biotin-containing peptides or biocytin. The biotin-lysine amide bond is not hydrolyzed by proteolytic enzymes or peptidases. A specific hydrolyase, biotinidase, catalyzes the cleavage of biocytin to biotin and lysine and of short biotinyl-peptides to biotin and peptides (82, 84, 153). This enzyme is found in the serum as well as in many tissues (175). One metabolic role for biotinidase is to release biotin from biocytin derived from proteolysis of holocarboxylases, permitting the reutilization of biotin. Most of the biotin in foods such as meat and cereals is protein-bound (63, 154) and biotinidase is the only known enzyme that catalyzes its release (170). Biotinidase is present in pancreatic juice and intestinal mucosa but is not enriched in intestinal brush-border membranes (168, 170). Thus it is likely that biotinidase plays an important role in the utilization of protein bound dietary biotin (170).

Very little is known about the mechanisms of absorption of biotin from the intestine. Everted sacs of rat intestine showed no concentrative uptake of biotin and a linear increase in movement across the intestine from 1–10 μM, which suggests diffusion (156). The concentration of biotin in rat intestinal contents was estimated to be 80–700 nM, and assuming rat plasma content of biotin to be comparable to the 0.8–3.0-nM biotin in human plasma, the concentration gradient would permit adequate uptake by diffusion. Another study of intestinal transport of biotin confirmed the lack of concentrative transport in the rat, rabbit, and guinea pig, but found concentrative transport in the mouse and hamster (137). Further study of the transport of biotin in the hamster small intestine showed it to be activated by sodium and to have a K_t for biotin of 1 mM (20). There is some question about whether this is the physiological mechanism for biotin transport since the K_t was so high and lipoic acid (thioctic acid) was a competitive inhibitor with a K_i one third the K_t for biotin. It has been shown in vivo in humans that, at high concentration, biotin can be absorbed in the large intestine as well as the small intestine (136). Even less is known about the absorption of biocytin, although it is a competitive inhibitor of biotin absorption in the small intestine of the hamster (137).

Some of the biotin in plasma is free but considerable amounts are bound (139). Biotin binds to crude fractions of human albumin and alpha- and beta-globulins (51). There is a biotin-binding glycoprotein in human plasma (54, 157). Whether this protein has a functional role in biotin transport is not

known. The clearance of biotin by the kidneys is 41–44% of that of the clearance of creatinine at normal concentrations of biotin in plasma (17). This may be due to incomplete glomerular filtration as a consequence of the binding of biotin to plasma proteins or to tubular reabsorption. Rat renal brush border vesicles show facilitated diffusion of biotin (17).

The cellular uptake of biotin by fully differentiated mouse 3T3-L1 cells (adipocyte-like) is temperature dependent, saturable at concentrations less than 50 μM, and relatively specific (34). However the K_m of 22 μM appears rather unphysiological since human plasma levels of biotin are about 10,000 times lower than this K_m. Above 50-μM biotin, the uptake was linear and nonsaturable, which suggests diffusion. Biotin uptake by HeLa cells in 20–400-μM biotin was temperature dependent but nonsaturable (39). In contrast, uptake of biotin in an avidin complex was temperature dependent, saturable, and greater than the uptake of free biotin. Similar results were found for human fibroblasts (30). It was suggested that the biotin-avidin complex was bound to the cells and taken up by pinocytosis. Since avidin is not a mammalian protein, they suggested that it mimics an as yet unknown biotin-binding plasma protein. There is considerable question about the physiological relevance of these studies since the lowest concentration of free biotin used was 0.8 μM, which is about 300 times the concentration of biotin in human plasma.

Dietary Requirement for Biotin

The dietary requirement for biotin in man is not known with certainty. This is partially a result of uncertainty in the analysis of available biotin in foods and of uncertainty in the magnitude of biotin production by intestinal flora. The methods for analyzing biotin and its nutritional importance have been reviewed (25, 163). Most analyses for biotin have been done with microbiological assays (163). Some microorganisms can utilize only biotin, whereas others can utilize biotin and biocytin. More recent radioisotope dilution assays with avidin measure any ureido-containing metabolite as well as biotin (40). Another variable in the analysis is that of the techniques used to digest the foods to release biotin. There is some uncertainty about how much of the microbiologically assayed biotin in different foods is actually available for mammalian nutrition.

The approximate biotin contents of foods have been tabulated (68, 163). Note that the concentrations in (163) should be in μg/100 g rather than mg/100 g. Liver, egg yolks, and cooked cereals are highest in biotin, containing 20–100 μg/100 g. The calculated biotin in a composite Canadian diet was 62 μg per day and actual analysis of the diet gave 60 μg per day (73). A similar calculation of the British diet revealed an intake of 33 μg of biotin per day (27). When daily dietary intakes were 33, 37, and 54 μg of biotin, the daily urinary excretions of biotin were 30, 51, and 42 μg respectively, and daily fecal excretions of biotin

were 79, 191, and 241 respectively (41, 52, 110). Thus urinary excretions of biotin were approximately equal to dietary input while fecal excretions were 2.5 to 5 times higher. This indicates considerable synthesis of biotin by the intestinal flora, but the amounts that are absorbed are unknown.

The extent of synthesis of biotin by the intestinal flora and the amount absorbed from the intestine have not been conclusively measured by attempts to sterilize the gut with antibiotics. Although fecal excretion of biotin decreased with oral administration of sulfonamides in one study (61), in another there was no decrease when avidin was not included in the diet (111). There was no decrease in the urinary excretion of biotin in these studies, nor was there a decrease when neomycin was given (93). In contrast, a very high dose of streptomycin caused a marked decrease in urinary biotin (130). It is possible that most of the biotin synthesized by intestinal flora is retained intracellularly as protein-bound biotin and that relatively little is available for human nutrition.

Typical dietary intakes of biotin are 30–60 μg per day (27, 73). The lack of any clinical indications of biotin deficiency at this level suggests that this is an adequate daily intake. European countries do not have a recommended intake of biotin, but in Canada 40 μg per day is the suggested intake (27) and in the United States intakes of 35 μg per day for infants, increasing with age to 200 μg per day, have been recommended (107). Human breast milk contains 0.7–1.3 μg of biotin per 100 ml or 0.17 μg per g of dry matter, similar to formulas based on whole cow's milk (0.21 μg per g dry matter); formulas based on skim milk or demineralized milk contains less biotin, a mean of 0.095 (72). Infants in the US who are breast fed or given formulas containing minimal biotin typically consume 6–10 μg of biotin per day (169). During mature milk production, 2–14% (1–11 μg per day) of the maternal intake of biotin (81 μg per day) was found in breast milk (36).

The human neonate begins extrauterine life with higher levels of biotin in the blood than those of adults. The concentration of biotin in cord blood is 35–50% higher than in maternal blood (3, 4). Oral or intramuscular administration of 200 μg of biotin to the mother 1–7 hours before birth significantly elevated biotin levels in both maternal and cord blood and increased the concentration in cord blood 100% above that in maternal blood (3). These studies indicate that placental transport of biotin occurs. In these studies, maternal blood levels of biotin were normal at the time of delivery. In another study maternal blood levels of biotin were about 50% of normal between 3 and 9 months of pregnancy (21). The intake of biotin from breast milk and the urinary output of biotin has been studied over the first week of life (67). Urinary biotin levels were higher from one to three days, then decreased five-fold by days six and seven. The intake of biotin was almost zero on days one and two then rose to a plateau by day six. Urinary excretion greatly exceeded intake from breast milk for the first four days, and at days six and seven intake was about double the excretion.

BIOTIN-RESPONSIVE MULTIPLE CARBOXYLASE DEFICIENCY

Isolated Deficiencies of Individual Carboxylases

Inherited deficiencies of each of the three mitochondrial biotin-containing carboxylases are known in humans. These isolated deficiencies are due to abnormal apoenzyme structures and do not respond to pharmacological doses of biotin.

Propionic acidemia is due to a deficiency of propionyl-CoA carboxylase. Patients with this disorder typically have episodic vomiting, severe ketosis, and metabolic acidosis, progressing to coma. If the disorder is not fatal in early infancy, many patients have failure to thrive and mental retardation (172). The characteristic biochemical abnormalities are elevated concentrations of propionic acid in blood and elevated levels of secondary metabolites in urine, including 3-hydroxypropionic acid, an oxidation product, and 2-methylcitric acid, which is formed by the condensation of propionyl-CoA and oxaloacetic acid in a reaction catalyzed by citrate synthetase. When patients are acutely ill, propionylglycine, tiglylglycine, 3-hydroxy-*n*-valeric acid, and other abnormal metabolites are found in the urine. In very young infants concentrations of ammonia are elevated in blood, as are concentrations of glycine in plasma and urine. Patients have been reported with biotin-responsive propionic acidemia (6, 71). In one, the concentrations of propionic acid in blood achieved after an isoleucine load appeared to be less after treatment with biotin (6), but the clinical course was not appreciably altered by treatment (J. V. Leonard, personal communication) and the activity of propionyl-CoA carboxylase in fibroblasts remained very low in the presence of high concentrations of biotin (L. Sweetman, unpublished). In the other (71), studies of peripheral lymphocyte carboxylases after a long period without biotin were completely normal and the patient was well (J. C. Williams and L. Sweetman, unpublished).

Isolated deficiency of 3-methylcrotonyl-CoA carboxylase has been reported in three patients. Two siblings were asymptomatic while receiving a low-protein diet (19). Increased dietary protein caused vomiting, acidosis, and hypoglycemia in one patient. There was some hair loss but no rash. Another patient presented with severe hypoglycemia, mild metabolic acidosis, hypotonia, and coma (8). All of the patients excreted large amounts of the metabolites of 3-methylcrotonyl-CoA: 3-hydroxyisovaleric acid (which is formed by hydration) and 3-methylcrotonylglycine (which is formed in a reaction catalyzed by glycine *N*-acylase) as well as variable amounts of 3-methylcrotonic acid. None of the patients responded to biotin with a decrease in the excretion of metabolites, but all were clinically well after treatment with diets restricted to 1.8–2.0 g of protein/kg body weight per day. A patient reported earlier with neurological symptoms similar to Werdnig-Hoffmann disease in whom 3-

methylcrotonylglycinuria and 3-hydroxyisovaleric aciduria were unresponsive to small doses of biotin may have had the same disorder, but multiple carboxylase deficiency was not totally excluded (138). This is true for another patient who excreted large amounts of 3-hydroxyisovaleric acid and was deficient in 3-methylcrotonyl-CoA carboxylase (49). In two additional patients with biotin-responsive excretion of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid, multiple carboxylase deficiency was not excluded (32, 56, 80).

Isolated deficiency of pyruvate carboxylase causes an elevation of lactic acid, pyruvic acid, and alanine levels in blood (42). There are two forms of this disorder, a more severe form that does not produce the pyruvate carboxylase protein and a less severe form that produces an enzymatically defective pyruvate carboxylase (123). These patients usually have severe neurological problems and early death. None of the patients have responded to biotin therapy.

A patient with an isolated deficiency of acetyl-CoA carboxylase has been reported (22) but there was not a consistent deficiency of the enzyme in cultured fibroblasts (H. R. Scholte, personal communication).

Abnormal Holocarboxylase Synthetase

The literature on biotin-responsive multiple carboxylase is confusing because of the evolution of terminology and knowledge. Most of the early studies by a variety of investigators were of a single patient, variously referred to as J.R. and J.Ri. Therefore it is useful to review the historical progression of the many studies on this patient that culminated in the characterization of an abnormal holocarboxylase synthetase.

J.R. was initially described in 1971 under the heading of biotin-responsive β-methylcrotonylglycinuria (58). He had tended to vomit since birth, and an erythematous skin rash began at six weeks of age. At five months he developed rapid respiration, persistent vomiting, and unresponsiveness, and was found to have ketosis and metabolic acidosis. Analysis of urine by gas chromatography–mass spectrometry showed a large elevation of 3-methylcrotonic acid and 3-methylcrotonylglycine, with lesser elevations of 3-hydroxyisovaleric acid and tiglylglycine (57, 58). The pattern of excretion of metabolites was largely consistent with a deficiency of 3-methylcrotonyl-CoA carboxylase. Reasoning that this was a biotin-requiring enzyme and that biotin might be therapeutic, an empirical dose of 10 mg of biotin per day was given orally. The clinical and biochemical response was dramatic. Vomiting, ketosis, and acidosis resolved in one day, the elevated levels of urinary metabolites became normal in 2–4 days. The skin rash cleared, and he developed normally with continued treatment with biotin. At two years of age, the dose of biotin was reduced until he began to excrete 3-methylcrotonylglycine and 3-hydroxyisovaleric acid. At that time the activity of 3-methylcrotonyl-CoA carboxylase in leucocytes was

shown to be low, 12% of an age-matched control or 20% of the normal adult level of activity (59).

Two facts suggested that an abnormal 3-methylcrotonyl-CoA carboxylase was not the complete story. First, a deficiency of this enzyme did not account for the excretion of tiglylglycine since tiglyl-CoA is not a normal substrate for the enzyme. A year after the initial report, tiglylglycine levels were shown to be elevated in urine of patients acutely ill with propionic acidemia in whom there is a deficiency of another biotin-containing enzyme, propionyl-CoA carboxylase (121). Second, free biotin is not a substrate or cofactor for 3-methylcrotonyl-CoA carboxylase itself, but rather a substrate for holocarboxylase synthetase, which attaches biotin covalently to apo-3-methylcrotonyl-CoA carboxylase.

Although the specificity of holocarboxylase synthetase for apocarboxylase was not known, it was thought likely that it acted on more than one apocarboxylase. Consequently, studies on the possibility of a biotin-responsive deficiency of propionyl-CoA carboxylase were undertaken. At two years of age, while J.R. was clinically well but receiving only 0.5 mg of biotin per day, he had a large excretion of 3-hydroxyisovaleric acid of about 7 μmol per mg creatinine, and about 30% as much 3-methylcrotonylglycine (143). There were very significant elevations in the excretion of 3-hydroxypropionic and 2-methylcitric acids, which are the characteristic urinary metabolites of patients with propionic acidemia. Reanalysis of the urines obtained at the time of the initial diagnosis also showed elevated concentrations of 3-hydroxypropionic acid and 2-methylcitric acid (31). The activity of propionyl-CoA carboxylase was assayed in fibroblasts derived from J.R., which were cultivated in Eagle's Minimal Essential Medium with 10% fetal calf serum. They showed only 4% of normal activity, a level similar to the 2% of normal activity obtained in patients with propionic acidemia (143). It was fortunate that the fibroblasts were grown in this medium in which the only biotin was that from the fetal calf serum (about 6 nM). In a subsequent study the activity of propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase was markedly deficient in this medium, but when the cells were cultivated in Hamm's F-10 medium, which contains about 100-nM biotin, the activities of both enzymes were in the normal range (160). Normal fibroblasts had the same activities of the carboxylases in both media.

When the patient's cells were cultivated in different concentrations of biotin, the activities of the two enzymes gave Michaelis-Menton curves with apparent K_m's for biotin of 60–90 nM (10). When protein synthesis was inhibited in the patient's fibroblasts that had been cultivated without added biotin, there was a large increase in carboxylase activity within three hours of the addition of biotin, which indicates that biotin was being attached to preexisting apocarboxylases (11). The biotin-responsiveness of the two carboxylases suggested that the primary defect was in holocarboxylase synthetase. The third mito-

chondrial carboxylase, pyruvate carboxylase, was also shown to be deficient and biotin responsive in the fibroblasts of J.R. (132). These observations suggested that a single holocarboxylase synthetase attaches biotin to all three mitochondrial apocarboxylases. That the same holocarboxylase synthetase also attaches biotin to the cytoplasmic apoacetyl-CoA carboxylase was indicated by the demonstration of biotin-responsive deficiencies of acetyl-CoA carboxylase in fibroblasts of J.R. and other patients (48, 113).

Holocarboxylase synthetase was assayed in cultured fibroblasts by using as substrate apopropionyl-CoA carboxylase purified 200-fold from rats made biotin deficient by a high-avidin (raw egg white) diet (28). Fibroblast extracts were incubated with excess rat apopropionyl-CoA carboxylase, ATP, and various concentrations of biotin, and the holopropionyl-CoA carboxylase formed was assayed by fixation of radioactive bicarbonate and propionyl-CoA to the acid nonvolatile radioactive product methylmalonyl-CoA. In contrast to the K_m for biotin of about 8 nM for normal fibroblasts, the patient's K_m for biotin was about 60-fold higher and the maximum velocity was 30–40% of normal. These kinetic properties of the mutant holocarboxylase synthetase were consistent with the response of the carboxylases in fibroblasts to various concentrations of biotin in the culture media. They were also consistent with the in vivo response of the patient to biotin.

Normal levels of biotin in plasma are 0.8–3.0 nanomolar, which may be similar to intracellular levels. These values are only slightly below the K_m of normal holocarboxylase synthetase and are sufficient for converting apocarboxylases to holocarboxylases. On the other hand, in the patient J.R. normal plasma levels of biotin would be about 1/170 of the K_m of his holocarboxylase synthetase, effectively preventing the conversion of apocarboxylases to holocarboxylases. Treatment with 10 mg of biotin per day, about 200 times the normal intake, elevates the plasma concentration of biotin above the K_m for his holocarboxylase synthetase, allowing conversion of apocarboxylases to holocarboxylases even though there is a somewhat decreased maximum velocity of holocarboxylase synthetase.

Many other patients with biotin-responsive multiple carboxylase deficiency have now been reported and a wide range of clinical symptoms have been observed. Some, such as an infant who died with 3-methylcrotonic aciduria and lactic acidemia (126), have been shown to have the same genetic defect as J.R. by lack of genetic complementation in fibroblast heterokaryons produced by cell fusion (132). Others have been shown to have an abnormal holocarboxylase synthetase by using endogenous lymphoblast or fibroblast apocarboxylases as substrates (9, 55, 131).

Fibroblasts from seven patients have been shown to have abnormal holocarboxylase synthetase activity using rat apopropionyl-CoA carboxylase as substrate and their clinical features are summarized (29).

The age of onset of clinical symptoms varied from the first day of life to eight months of age, but most patients presented before six weeks of age. Initially it had been thought that two forms of biotin-responsive multiple carboxylase deficiency could be differentiated by the age of onset. Those with holocarboxylase synthetase abnormalities generally presented within the first six weeks of life and were called the early-onset or neonatal multiple carboxylase deficiency (142). The form that generally presented after six months of age, now known to be due to a deficiency of biotinidase, was referred to as late-onset, late-infantile, or juvenile multiple carboxylase deficiency. It is now known that patients with an abnormal holocarboxylase synthetase can present at any age from one day to 15 months (135).

There may be some correlation of the age of onset with the degree of elevation of the K_m for biotin of holocarboxylase synthetase (29). The patient with the highest K_m for biotin (70 times normal) presented in the first few hours of life (171) and a previous sibling had died at three days of age (144). Patients in whom the K_m values for biotin of holocarboxylase synthetase were 20 to 45 times normal presented between one day of life and seven weeks. The patient with a K_m for biotin only three times normal presented at eight months of age (113). That holocarboxylase synthetase deficiency is a serious life-threatening disorder is indicated by the occurrence of death in the neonatal period in three patients (126, 144). In two of those the diagnosis was based on the presence of the characteristic organic aciduria in samples studied postmortem (144). The brother of one of these patients was documented to have the enzyme defect (29, 171).

Both males and females are affected with holocarboxylase synthetase abnormality, and the inheritance appears to be autosomal recessive. One patient was asymptomatic in whom treatment with biotin had been undertaken pre- and postnatally (114), but a previous sibling had presented with the typical clinical and biochemical features on the second day of life (115). Common clinical features were hypotonia, developmental delay or regression, rash, and alopecia. Typical biochemical features were hyperammonemia, metabolic acidosis, lactic acidemia, and the presence of the characteristic urinary metabolites. The pattern of the organic aciduria includes a large elevation of the level of 3-hydroxyisovaleric acid, a smaller and variable elevation of the amount of 3-methylcrotonylglycine, and lesser amounts of 3-hydroxypropionic acid and 2-methylcitric acid. All of the patients showed marked clinical and biochemical improvement after treatment with biotin. Most of the patients became clinically normal after receiving 10 mg of biotin per day and had no elevation of the urinary levels of metabolites.

Systematic studies to determine whether lower doses of biotin would be sufficient have not been done. The original patient J.R. had no elevation in the levels of urinary metabolites while he was receiving 10 mg of biotin per day

(58) but he did have significant amounts when the dose was 0.5 mg per day (143). Some patients, although clinically well when given 10 mg of biotin per day, had small amounts of urinary metabolites that became normal when the dose was 40 mg of biotin per day (114, 115). Another less responsive patient was clinically well when receiving 1 to 20 mg of biotin per day, but had elevated excretions of metabolites of leucine and activities of the carboxylases in leucocytes that were only 4 to 16% of normal when receiving 20 mg of biotin per day (106). The patient with the highest known K_m for biotin (29) continued to have a skin rash, large excretions of metabolites, and subnormal activities of carboxylases in lymphocytes when receiving doses of biotin as high as 60 mg per day (29, 171). When provided with adequate biotin therapy, none of the patients have required dietary restriction of protein, although moderate restriction in the less-responsive patients could well decrease the excretion of metabolites as it does for patients with isolated biotin-unresponsive propionic acidemia (172) and 3-methylcrotonyl-glycinuria (8, 19).

Patients with biotin-responsive multiple carboxylase deficiency due to an abnormal holocarboxylase synthetase share a basic distinguishing biochemical feature: carboxylases have low activity in fibroblasts cultivated in medium containing biotin only in the amounts provided by the fetal calf serum, and they increase in activity when the medium is supplemented with high concentrations of biotin (29). In contrast, fibroblasts from patients with biotin-responsive multiple carboxylase deficiency that is due to a deficiency of biotinidase have normal activities of the carboxylases in both types of media (29). These criteria can be used to identify the patients in the literature with an abnormal holocarboxylase synthetase even if the synthetase enzyme has not been assayed directly (13, 14, 92, 112). Heterozygotes cannot be distinguished by this means but neither can they be detected by current assays of holocarboxylase synthetase. A rapid diagnostic method for distinguishing holocarboxylase synthetase abnormalities from biotinidase deficiency is the assay of the activity of carboxylases in lymphocytes isolated from blood that has or has not been preincubated with biotin (140). Assay of levels of biotin may be helpful, and of course assay of biotinidase is now a simple way of detecting that defect (140, 164).

An abnormal holocarboxylase synthetase can be diagnosed prenatally by demonstrating biotin-responsive deficiencies of carboxylases in cultured amniocytes obtained by amniocentesis (114). The prenatal diagnosis in this pregnancy was also based on the demonstration of a small but significant amount of 2-methylcitric acid in the amniotic fluid, as measured by stable isotope dilution. A much more significantly elevated concentration of 3-hydroxyisovaleric in the amniotic fluid was later shown by stable isotope dilution gas chromatography–mass spectrometry, while normal levels were found in a subsequent unaffected pregnancy (76). This appears to be the best method for the rapid prenatal diagnosis of this disorder.

Prenatal therapy was begun at 23.5 weeks of pregnancy by giving the mother 10 mg of oral biotin per day. This greatly increased her serum level of biotin and there were no ill effects (114). At birth the infant was clinically well and levels of urinary organic acids were normal. The prenatal diagnosis was confirmed by assay of holocarboxylase synthetase in cultured skin fibroblasts (29). The baby has remained asymptomatic while receiving 40 mg of biotin per day (114).

Prenatal therapy was also carried out without prior diagnosis at 34 weeks in a pregnancy at risk for an abnormal holocarboxylase synthetase (127). Clinically well fraternal twins were born with concentrations of biotin in cord blood 4–7 times normal and with normal urinary organic acids. Postnatal therapy with biotin was not given while fibroblasts were being cultured to establish a diagnosis; the twin who was affected presented at three months of age moribund, hypothermic, and in shock (128). He was severely acidotic and ketotic and had elevated levels of lactic and pyruvic acids in blood and large elevations in the concentrations of urinary metabolites. Treatment with 10 mg of biotin per day was effective and resulted in rapid clinical and biochemical improvement (125, 128).

Biotinidase Deficiency

The patients who were initially designated as having "late-onset" biotin-responsive multiple carboxylase deficiency were shown by Wolf and colleagues in 1983 to have a deficiency of biotinidase in serum (164, 167). This clarified many of the problems in the earlier literature about the biochemistry of this disorder. Typically, patients with a deficiency of biotinidase have presented after three months of age with symptoms like those of patients with an abnormal holocarboxylase synthetase. These include hypotonia, developmental delay or regression, skin rash, and alopecia. They may present with life-threatening episodes of metabolic acidosis and this may be complicated by hyperammonemia. More specific abnormalities include lactic acidemia and the characteristic organic aciduria consisting of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, 3-hydroxypropionic acid and 2-methylcitric acid (29, 165). In addition many of the patients have had seizures, ataxia, candidiasis, and conjunctivitis. One patient had the cutaneous and neurological symptoms but an organic aciduria was not detected (146). This can be a fatal disorder. Two previous siblings who had had clinical symptoms similar to a diagnosed patient had died at 8 and 39 months of age (37, 161). The three infants in this family had defects in T-cell and B-cell immunity (37). The diagnosed patient had pretreatment levels of biotin in plasma that were somewhat below normal (116) as did other patients without abnormal T and B cells (104, 150). Another patient had fatty acid and biotin-responsive impairment of lymphocyte suppressive activity in vitro (50). Biotin deficiency in guinea pigs has been shown to decrease the numbers of B and T lymphocytes (120).

Some patients with biotinidase deficiency have had optic atrophy (124, 165) or neurosensory hearing loss (165, 166). A patient diagnosed at 10 months of age and treated with 10 mg of biotin per day (33) was subsequently shown to have a deficiency of biotinidase (12) and developed a sensorineural hearing loss and severe myopia suggestive of a progressive retinal epithelial dysplasia (149). None of the patients with an abnormal holocarboxylase synthetase have developed these neurosensory abnormalities, ruling out long-term treatment with biotin as a cause in the patients with biotinidase deficiency. The alopecia and skin rash in one patient with biotinidase deficiency responded to oral and cutaneous administration of unsaturated fatty acids (105). This suggests that a deficiency of acetyl-CoA carboxylase required for fatty acid synthesis may be involved in the pathogenesis of these symptoms.

Patients deficient in biotinidase usually respond to 10 mg of oral biotin per day, with reversion to normal of all of the biochemical and clinical features of the disease except for hearing loss and optic atrophy (33, 37, 104, 116, 150, 164, 165). Fibroblasts cultured from patients with biotinidase deficiency have normal activities of the carboxylases (13, 92, 94, 112, 116, 150). This is because there is sufficient biotin in fetal calf serum so that recycling of biocytin by biotinidase in the cells is unnecessary; in addition, fetal calf serum has biotinidase activity. Holocarboxylase synthetase exhibits normal kinetics in these cells (29).

Before the identification of biotinidase deficiency as the defect in "late-onset" biotin-responsive multiple carboxylase deficiency, there was speculation about a defect in the intestinal absorption of biotin since pretreatment levels of biotin in plasma and urine were often low (104, 116, 150). This was studied by administering small oral doses of biotin to patients with below normal plasma levels of biotin, and measuring the rise in plasma biotin compared to normals (103, 151). The less than normal rise in plasma biotin in the patients was attributed to defective intestinal absorption. With the discovery that biotinidase was deficient, a similar study was repeated after first treating the patient with enough biotin to raise the levels in plasma to somewhat above normal (152). Under these conditions, the rise in plasma biotin following a small oral load was normal, which indicates normal intestinal absorption. Apparently, in the previous studies the tissues were so depleted of biotin that absorbed biotin was rapidly removed from the plasma, preventing a normal rise in plasma levels.

In the biotin-deficient state, an increase in renal clearance of biotin was observed (151), but this became normal upon repletion with biotin (152). In another study, a normal rise in plasma biotin was found in a biotin-deficient patient (18). When plasma levels of biotin were normal, renal clearance of biotin was elevated in this patient. A more extensive study of five patients with biotinidase deficiency confirmed a renal clearance of biotin increased 2–3 times

above normal when plasma biotin levels were normal (16, 17). Upon cessation of treatment with 10 mg of biotin per day, the patients had a more rapid fall in plasma biotin and a greater renal loss of biotin than did controls.

Biocytin was detected in the urine of six patients with biotinidase deficiency but not in normal urine (26). The levels of biocytin were considerably higher than the levels of biotin when the patients were not receiving biotin. The normal renal clearance of biotin is half that of creatinine, and if this reflects renal reabsorption, the increased clearance of biotin in biotinidase deficiency might be due to an inhibition of biotin reabsorption by the elevated amounts of biocytin. The clinical and biochemical features of biotinidase deficiency appear to be related to deficiencies of the carboxylases secondary to the functional deficiency of biotin. Some patients have deficient levels of biotin plasma while others have normal levels. Biocytin may compete with biotin as a substrate for holocarboxylase synthetase, thus increasing the concentration of biotin needed for effective holocarboxylase synthesis. It is possible that elevated levels of biocytin are toxic but this has not been studied experimentally.

Biotinidase deficiency is readily demonstrable by assay of the enzyme in serum (164, 167). Parents of affected infants have about 50% of normal activity, consistent with heterozygosity and autosomal recessive inheritance. The enzyme has also been shown to be deficient in the liver of a deceased patient (53). Biotinidase can be measured in a colorimetric assay using the artificial substrate, biotinyl-*p*-aminobenzoic acid (164). A more sensitive assay employs radioactively labeled biotinyl-^{14}C-*p*-aminobenzoic acid (173). A small amount of biotinidase activity was detectable in normal fibroblasts while none was found in patient fibroblasts. This assay also detected biotinidase activity in normal amniocytes, which suggests that prenatal diagnosis of biotinidase deficiency may be possible (134). A fluorometric assay with an artificial substrate for biotinidase has been described (158). A radiochemical assay with the natural substrate ^{14}C-*d*-biotinyl-ε-*N*-L-lysine has also been described (90).

The colorimetric assay for biotinidase activity has been adapted in a low-cost screening method for newborns, utilizing the dried blood samples spotted on filter papers for newborn screening for phenylketonuria (70). This was recently used to screen 81,243 newborns in Virginia, among whom two unrelated patients were diagnosed as having biotinidase deficiency (169). Two older, previously undiagnosed siblings with severe neurological abnormalities, developmental delay, and cutaneous symptoms were found by assay of the families of the two infants positive on the screening assay. From this study the incidence of biotinidase deficiency was estimated to be 1 in 40,000, but the ultimate incidence may be between 1 in 12,000 and 1 in 240,000 births. Biotinidase deficiency fits the accepted criteria for inclusion in mass newborn screening programs: it has a significant incidence, it is not easily recognized clinically before serious symptoms occur, it is life-threatening, treatment with

biotin is very effective, and a low-cost screening method is available. It would be very worthwhile to make screening for biotinidase deficiency widely available.

DIETARY DEFICIENCY OF BIOTIN

The literature on the dietary deficiency of biotin in man is small. Dietary deficiency appears to be rare as a result of the general availability of biotin in the diet and the small requirement for biotin. The synthesis of biotin by intestinal bacteria may be a factor. The early instances of biotin deficiency were all a consequence of diets containing large amounts of raw egg white in which avidin prevented intestinal absorption of biotin. In an experimental study, biotin deficiency was induced in four adult volunteers by using a diet in which raw egg white made up 30% of the caloric intake (147, 148). The subjects developed glossitis, anorexia, nausea, and an active distaste for the diet. Behavioral manifestations included depression, hallucinations, somnolence, and a panic state. A desquamating dermatosis developed. All of the symptoms were reversed by daily injection of 150 μg of biotin. Since this study was done before the biochemical role of biotin in metabolism was understood, no biochemical data were obtained other than levels of biotin, which decreased markedly in the urine while on the diet.

In contrast to this study patients with malignancies given equally large amounts of egg white and/or avidin in two studies did not develop clinical symptoms (79, 122). When urinary biotin was measured it did not decrease while the patients were receiving the diet (122).

Several patients have been reported in whom biotin deficiency developed as a consequence of unusual dietary habits that included ingestion of raw egg white. The first patient, a 66-year-old male, had for the previous six years consumed 3–10 raw eggs and 1–4 quarts of wine per day as well as very limited quantities of food (162). He had an erythematous exfoliative dermatitis for several years and conjunctivitis for several months. Histology of the skin showed an absence of sebaceous glands and atrophic hair follicles. Serum concentration of biotin was below normal but urinary biotin was normal. When placed on a normal hospital diet, even supplemented with his usual six raw eggs and a quart of wine per day, the skin rash resolved although the serum biotin remained below normal. Treatment with 500 μg of biotin injected on alternate days brought his serum levels of biotin to normal.

A 62-year-old female with cirrhosis was prescribed a regimen of six raw eggs daily for 18 months (15). She developed anorexia, nausea, vomiting, pallor, depression, lassitude, substernal pain, and a scaly dermatitis and desquamation of the lips. All of her symptoms were markedly improved by 2–5 days of the parenteral administration of 200 μg of biotin per day while continuing the raw

egg diet. Prior to treatment, blood levels of biotin were low (250 ng/l; normal 820–2700) but urinary biotin was normal.

A 5-year-old boy requiring tube-feeding received six raw eggs daily as well as antibiotics (133). He developed a fine scaly dermatitis and severe alopecia after 18 months. Laboratory findings were normal except for hypercholesterolemia. On substituting egg yolks without egg whites and administering 2–4 mg of biotin parenterally and by mouth, the rash disappeared, the hair grew, and the serum cholesterol dropped. On further treatment without biotin but with cooked eggs, there were no clinical symptoms but cholesterol rose. In a similar situation an 11-year-old boy was given a diet of infant formula containing oil and two raw eggs per day (145). He developed a severe erythematous exfoliative dermatosis and total alopecia. Blood cholesterol was not elevated. Plasma concentration of biotin was low (156 ng/l; normal 330–722) as was urinary biotin (0.85–1.31 ng/mg creatinine; normal 11–95). He had the typical organic aciduria seen in biotin-responsive multiple carboxylase deficiency in which there are elevated quantities of 3-methylcrotonylglycine, 3-hydroxyisovaleric acid, 3-hydroxypropionic acid, and 2-methylcitric acid. In addition he had intermittent ketosis. The activities of propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase in leucocytes were low (2% and 10% of normal, respectively). All of the biochemical and clinical manifestations resolved on substituting cooked eggs and providing 1 mg of intravenous and later oral biotin.

It is interesting that biotin deficiency occurred in these patients who received whole raw eggs. Egg yolks contain large amounts of biotin, slightly more than one would think would be bound by the avidin in the egg whites. Whole raw eggs do not cause symptomatic biotin deficiency in experimental animals. Factors that may have rendered these humans more susceptible to biotin deficiency include cirrhosis and age in the adults and chronic oral antibiotics in the children.

Milder forms of biotin deficiency may occur, manifested by apparent seborrheic dermatitis or Leiner's disease in infants (108). A number of studies have reported rapid clearing of the dermatitis with biotin treatment (98, 108, 141), but in one double-blind study biotin was ineffective (47). It is possible that different types of patients were studied in the different studies. Understanding of the possible role of biotin in seborrheic dermatitis is hampered by the lack of measurement of biotin levels in most of the studies and the total lack of other biochemical measurements such as leucocyte carboxylase activities or urinary organic acids (7).

Of clinical relevance are reports of frank biotin deficiency in patients receiving total parenteral nutrition (TPN). Many intravenous preparations do not contain biotin, and it had also been overlooked in regimens of TPN. An infant with short-gut syndrome receiving continuous antibiotics developed an ery-

thematous rash after three months of TPN (101). After five months, total alopecia occurred with pallor, irritability, lethargy, and mild hypotonia. Essential fatty acids were not deficient and zinc supplementation was ineffective. The concentration of lactic acid was elevated in blood, and analysis of urinary organic acids showed the characteristic organic aciduria of biotin deficiency. Plasma and urinary concentrations of biotin were deficient. On treatment with 10 mg of biotin per day in the parenteral fluid, the biochemical and clinical manifestations reverted to normal. When the patient was given a maintenance dose of 100 μg of biotin per day for nine months, the manifestations did not reappear.

Two siblings with congenital secretory diarrhea and seizures developed similar clinical and biochemical abnormalities on total parenteral nutrition (81). Urinary biotin levels were low but serum biotin was normal. One child died with metabolic acidosis. In the other sibling 200 μg of intravenous biotin per day brought to normal the organic aciduria but not the skin lesions, which required 10 mg of biotin per day for resolution. An adult patient receiving home parenteral nutrition developed skin lesions, alopecia, acidosis, and neurological findings that resolved on the administration of 60 μg per day of intravenous biotin (96). Two adults receiving long-term parenteral nutrition developed severe alopecia that was reversed by 200 μg per day of biotin (74). Extensive study of three children with biochemical and clinical symptoms of biotin deficiency as a result of total parenteral nutrition revealed that all three had markedly depressed excretion of biotin but that only one had a low plasma level of biotin (99, 100). In the two infants treated with 100 μg of intravenous biotin per day, there was gradual clinical and biochemical improvement, but in one, urinary organic acids were still elevated after ten weeks of therapy. The response of these infants suggests that 100 μg of biotin per day is adequate for maintenance, but is inadequate to replenish rapidly the depleted levels of biotin. Thus the recommendation of the Nutritional Advisory Group of the American Medical Association for 20 μg of biotin per day for children on parenteral alimentation (2) should be increased.

Biotin deficiency may also occur as a result of chronic hemodialysis. Three patients with encephalopathy and peripheral neuropathy and one with peripheral neuropathy all showed marked improvement within three months of the daily administration of 10 mg of biotin per day (176). Although biotin levels were not reported, it is likely that biotin deficiency can occur in chronic hemodialysis because of the loss of free biotin in the dialysate.

Another potential cause of biotin deficiency is the long-term administration of anticonvulsant medications. Patients receiving phenytoin, pyrimidone, phenobarbital, or carbamazepine (but not those receiving sodium valproate) had significantly lower plasma concentrations of biotin (227 ± 80 vs 448 ± 201 ng/l) (85, 86). In addition small elevations of the urinary levels of organic acids

characteristic of biotin deficiency were found (87, 88). The mechanism by which the anticonvulsant medications lower levels of biotin is unknown, but it may be related to effects on the intestinal absorption or renal loss of biotin. The drugs all contain a carbamide group, as does biotin.

A syndrome in young poultry, known as fatty liver and kidney syndrome, causes sudden unexpected death from hypoglycemia after stress. It may be due to moderate deficiency of biotin although it occurs without other symptoms of biotin deficiency (5, 119). This observation led to the investigation of a possible similar etiology in the sudden infant death syndrome (SIDS) in humans. The median level of free biotin in 35 infants who died of SIDS was 336 ng/g of autopsy liver, significantly lower than the mean of 419 ng/g for 57 infants with explainable deaths (77, 78). Although this observation suggests that a marginal deficiency of biotin (together with stress, by analogy to the syndrome in poultry) may be related to sudden infant death syndrome, more direct biochemical evidence such as assay of pyruvate carboxylase activity in autopsy liver or organic acids in urine or tissues is needed.

CONCLUSION

Considerable knowledge has been gained about the metabolism of biotin and its important role in humans through the elucidation of the biochemical abnormalities in the inherited human disorders that lead to biotin-responsive multiple carboxylase deficiency. Abnormalities have been studied in the two known enzymes of biotin metabolism, holocarboxylase synthetase and biotinidase. The biochemical abnormalities can be readily understood as the result of deficient activity of the biotin-containing carboxylases. In the presence of an abnormal holocarboxylase synthetase, in which there is an elevated K_m for biotin, the biochemical response to large doses of biotin reflects the elevation of cellular biotin levels to the range of the elevated K_m's. In biotinidase deficiency the carboxylase activities are low as a result of biotin deficiency and the response to biotin results from correcting this deficiency. The variable age of onset and clinical features of biotinidase deficiency probably reflect differences in the development of the biotin deficiency as a consequence of variations in the intake of free biotin.

The clinical abnormalities seen in these disorders are not all so clearly understood. The hair loss and skin rash may be related to a deficiency of acetyl-CoA carboxylase, required for fatty acid synthesis, because isolated deficiencies of the other three carboxylases have not been shown to result in these manifestations. The cause of the neurological abnormalities is even less obvious, but may be related to a deficiency of pyruvate carboxylase in brain (129). It is not known whether the probable elevation of biocytin in biotinidase deficiency has any biochemical or clinical effects. If biocytin does compete

with biotin as the substrate for holocarboxylase synthetase or for transport of biotin, treatment with doses of biotin much larger than needed to correct the biotin deficiency should minimize competition from biocytin.

The optimal dose of biotin for treatment has not been determined. In biotinidase deficiency, the same dose should be effective for all patients, and all would be expected to respond to biotin. With abnormalities of holocarboxylase synthetase, the required dose of biotin should be determined for each patient and will differ because there are differences in the degree of elevation of the K_m for biotin and in the maximum velocity. It is probable that non-K_m variants with deficient holocarboxylase synthetase activity exist that would not be responsive to biotin. These are likely to have considerable residual activity, as a complete deficiency would cause a complete deficiency of all four carboxylases and this would probably be incompatible with uterine development and life. No patients have been identified with biotin-responsive defects in the intestinal absorption of biotin, defects in plasma biotin-binding protein, defects of transport into cells, or defects in the renal handling of biotin. However, it is likely that these exist and will be encountered in the future.

The possibility of subclinical deficiencies of biotin exists in larger populations and may manifest only in susceptible or stressed individuals. Biotin deficiency in total parenteral nutrition should not be a problem now that adequate amounts of biotin are routinely added. Possible deficiency of biotin in patients receiving long-term hemodialysis should be determined. Marginal deficiencies of biotin in formula-fed infants should be eliminated by the fortification of formulas with larger amounts of biotin. The existence of lower levels of biotin in patients receiving long-term treatment with anticonvulsant medications is of concern, and other drugs should be investigated for possible similar effects. Treatment with moderate amounts of biotin would seem appropriate in long-term anticonvulsant therapy. The role of a marginal biotin deficiency as one possible cause of sudden infant death syndrome deserves further investigation.

Literature Cited

1. Achuta Murthy, P. N., Mistry, S. P. 1972. Synthesis of biotin-dependent carboxylases from their apoproteins and biotin. *J. Sci. Ind. Res.* 31:554–63
2. American Medical Association. 1979. Multivitamin preparations for parenteral use. A statement by the nutrition advisory board. *J. Parenter. Enteral Nutr.* 3:258–62
3. Baker, H., Frank, O., DeAngelis, B., Feingold, S., Kaminetzky, H. A. 1981. Role of placenta in maternal-fetal vitamin transfer in humans. *Am. J. Obstet. Gynecol.* 141:792–96
4. Baker, H., Thind, I. S., Frank, O., DeAngelis, B., Caterini, H., Louria, D. B. 1977. Vitamin levels in low-birth-weight newborn infants and their mothers. *Am. J. Obstet. Gynecol.* 129:521–24
5. Bannister, D. W. 1979. Recent advances in avian biochemistry: The fatty liver and kidney syndrome. *Int. J. Biochem.* 10: 193–99
6. Barnes, N. D., Hull, D., Balgobin, L., Gompertz, D. 1970. Biotin-responsive propionicacidaemia. *Lancet* 2:244–45
7. Barness, L. A. 1972. Letter: Treatment of seborrheic dermatitis with biotin

and vitamin B complex. *J. Pediatr.* 81: 631

8. Bartlett, K., Bennett, M. J., Hill, R. P., Lashford, L. S., Pollitt, R. J., Worth, H. G. J. 1984. Isolated biotin-resistant 3-methylcrotonyl CoA carboxylase deficiency presenting with life-threatening hypoglycaemia. *J. Inherit. Metab. Dis.* 7:182
9. Bartlett, K., Ghneim, H. K., Stirk, H.-J., Wastell, H. 1985. Enzyme studies in biotin-responsive disorders. *J. Inherit. Metab. Dis.* 8(Suppl. 1):46–52
10. Bartlett, K., Gompertz, D. 1976. Combined carboxylases defect: Biotin-responsiveness in cultured fibroblasts. *Lancet* 2:804
11. Bartlett, K., Gompertz, D. 1978. Biotin activation of carboxylase activity in cultured fibroblasts from a child with a combined carboxylase defect. *Clin. Chim. Acta* 84:399–401
12. Bartlett, K., Leonard, J. V. 1984. Letter: Phenotypic variability in biotinidase deficiency. *J. Pediatr.* 104:965
13. Bartlett, K., Ng, H., Dale, G., Green, A., Leonard, J. V. 1981. Studies on cultured fibroblasts from patients with defects of biotin-dependent carboxylation. *J. Inherit. Metab. Dis.* 4:183–89
14. Bartlett, K., Ng, H., Leonard, J. V. 1980. A combined defect of three mitochondrial carboxylases presenting as biotin-responsive 3-methylcrotonylglycinuria and 3-hydroxyisovaleric aciduria. *Clin. Chim. Acta* 100:183–86
15. Baugh, C. M., Malone, J. H., Butterworth, C. E. 1968. Human biotin deficiency. A case history of biotin deficiency induced by raw egg consumption in a cirrhotic patient. *Am. J. Clin. Nutr.* 21:173–82
16. Baumgartner, E. R., Suormala, T., Wick, H., Bausch, J., Bonjour, J.-P. 1985. Biotinidase deficiency: Factors responsible for the increased biotin requirement. *J. Inherit. Metab. Dis.* 8(Suppl. 1):59–64
17. Baumgartner, E. R., Suormala, T., Wick, H., Bausch, J., Bonjour, J.-P. 1985. Biotinidase deficiency associated with renal loss of biocytin and biotin. See Ref. 38, pp. 272–87
18. Baumgartner, E. R., Suormala, T., Wick, H., Geisert, J., Lehnert, W. 1982. Infantile multiple carboxylase deficiency: evidence of normal intestinal absorption but renal loss of biotin. *Helv. Paediatr. Acta* 37:499–502
19. Beemer, F. A., Bartlett, K., Duran, M., Ghneim, H. K., Wadman, S. K., et al. 1982. Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase in two sibs. *Eur. J. Pediatr.* 138:351–54
20. Berger, E., Long, E., Semenza, G. 1972. The sodium activation of biotin absorption in hamster small intestine *in vitro*. *Biochim. Biophys. Acta* 255:873–87
21. Bhagavan, H. N. 1969. Biotin content of blood during gestation. *Int. J. Vitam. Res.* 39:235–37
22. Blom, W., Scholte, H. R. 1981. Acetyl-CoA carboxylase deficiency: An inborn error of de novo fatty acid synthesis. *N. Engl. J. Med.* 305:465–66
23. Boas, M. A. 1924. LVIII. An observation on the value of egg-white as the sole source of nitrogen for young growing rats. *J. Biol. Chem.* 18:422–24
24. Boas, M. A. 1927. XCV. The effect of desiccation upon the nutritive properties of egg-white. *Biochem. J.* 21:712–24
25. Bonjour, J.-P. 1977. Biotin in man's nutrition and therapy—a review. *Int. J. Vitam. Nutr. Res.* 47:107–18
26. Bonjour, J.-P., Bausch, J., Suormala, T., Baumgartner, E. R. 1984. Detection of biocytin in urine of children with congenital biotinidase deficiency. *Int. J. Vitam. Nutr. Res.* 54:223–31
27. Bull, N. L., Buss, D. H. 1982. Biotin, pantothenic acid and vitamin E in the British household food supply. *Hum. Nutr. Appl. Nutr.* 36A:190–96
28. Burri, B. J., Sweetman, L., Nyhan, W. L. 1981. Mutant holocarboxylase synthetase: Evidence for the enzyme defect in early infantile biotin-responsive multiple carboxylase deficiency. *J. Clin. Invest.* 68:1491–95
29. Burri, B. J., Sweetman, L., Nyhan, W. L. 1985. Heterogeneity of holocarboxylase synthetase in patients with biotin-responsive multiple carboxylase deficiency. *Am. J. Hum. Genet.* 37:326–37
30. Chalifour, L. E., Dakshinamurti, K. 1982. The biotin requirement of human fibroblasts in culture. *Biochem. Biophys. Res. Commun.* 104:1047–53
31. Chalmers, R. A., Lawson, A. M., Watts, R. W. E. 1974. Studies on the urinary acidic metabolites excreted by patients with β-methylcrotonylglycinuria, propionic acidaemia and methylmalonic acidaemia, using gas-liquid chromatography and mass spectrometry. *Clin. Chim. Acta* 52:43–51
32. Chalmers, R. A., Spellacy, E. 1980. Biotin responsive 3-methylcrotonylglycinuria with normal carboxylase activities *in vitro:* Demonstration of a metabolic defect in the metabolism of isovalerate with a new assay. *Clin. Sci.* 58:18P–19P

33. Charles, B. M., Hosking, G., Green, A., Pollitt, R., Bartlett, K., Taitz, L. S. 1979. Biotin-responsive alopecia and developmental regression. *Lancet* 2:118–20
34. Cohen, N. D., Thomas, M. 1982. Biotin transport into fully differentiated 3T3-Ll cells. *Biochem. Biophys. Res. Commun.* 108:1508–16
35. Confalone, P. N., Pizzolato, G., Baggiolini, E. G., Lollar, D., Uskoković, M. R. 1975. A stereospecific total synthesis of *d*-biotin from L-(+)-cysteine. *J. Am. Chem. Soc.* 97:5936–38
36. Coryell, M. N., Harris, M. E., Miller, S., Rutledge, M. M., Williams, H. H., Macy, I. G. 1947. Metabolism of women during the reproductive cycle. XV. The utilizatin of biotin during lactation. *J. Lab. Clin. Med.* 32:1462–68
37. Cowan, M. J., Wara, D. W., Packman, S., Ammann, A. J., Yoshino, M., et al. 1979. Multiple biotin-dependent carboxylase deficiencies associated with defects in T-cell and B-cell immunity. *Lancet* 2:115–18
38. Dakshinamurti, K., Bhagavan, H. N., eds. 1985. *Biotin.* New York: NY Acad. Sci. 435 pp.
39. Dakshinamurti, K., Chalifour, L. E. 1981. The biotin requirement of HeLa cells. *J. Cell. Physiol.* 107:427–38
40. Dakshinamurti, K., Landman, A. D., Ramamurti, L., Constable, R. J. 1974. Isotope dilutin assay for biotin. *Anal. Biochem.* 61:225–31
41. Denko, C. W., Grundy, W. E., Porter, J. W., Berryman, G. H. 1946. The excretion of B-complex vitamins in the urine and feces of seven normal adults. *Arch. Biochem.* 10:33–40
42. DeVivo, D. C., Haymond, M. W., Leckie, M. P., Bussmann, Y. L., McDougal, D. B., Pagliara, A. S. 1977. The clinical and biochemical implications of pyruvate carboxylase deficiency. *J. Clin. Endocrinol. Metab.* 45:1281–96
43. DU Vigneaud, V., Melville, D. B., Folkers, K., Wolf, D. E., Mozingo, R., et al. 1942. The structure of biotin: A study of desthiobiotin. *J. Biol. Chem.* 146:475–85
44. DU Vigneaud, V., Melville, D. B., György, P., Rose, C. S. 1940. On the identity of vitamin H with biotin. *Science* 92:62–63
45. Eakin, R. E., Snell, E. E., Williams, R. J. 1940. A constituent of raw egg white capable of inactivating biotin in vitro. *J. Biol. Chem.* 136:801–2
46. Eisenberg, M. A. 1972. Biotin: biogenesis, transport, and their regulation. *Adv. Enzymol.* 36:317–72
47. Erlichman, M., Goldstein, R., Levi, E., Greenberg, A., Freier, S. 1981. Infantile flexural seborrhoeic dermatitis. Neither biotin nor essential fatty acid deficiency. *Arch. Dis. Child.* 56:560–62
48. Feldman, G. L., Wolf, B. 1981. Deficient acetyl CoA carboxylase activity in multiple carboxylase deficiency. *Clin. Chim. Acta* 111:147–51
49. Finnie, M. D. A., Cottrall, K., Seakins, J. W. T., Snedden, W. 1976. Massive excretion of 2-oxoglutaric acid and 3-hydroxyisovaleric acid in a patient with a deficiency of 3-methylcrotonyl-CoA carboxylase. *Clin. Chim. Acta* 73:513–19
50. Fischer, A., Munnich, A., Saudubray, J. M., Mamas, S., Coudé, F. X., et al. 1982. Biotin-responsive immunoregulatory dysfunction in multiple carboxylase deficiency. *J. Clin. Immun.* 2:35–38
51. Frank, O., Luisada-Opper, A. V., Feingold, S., Baker, H. 1970. Vitamin binding by humans and some animal plasma proteins. *Nutr. Rep. Int.* 1:161–68
52. Gardner, J., Parsons, H. T., Peterson, W. H. 1945. Human biotin metabolism on various levels of biotin intake. *Arch. Biochem.* 8:339–48
53. Gaudry, M., Munnich, A., Ogier, H., Marsac, C., Marquet, A., et al. 1983. Deficient liver biotinidase activity in multiple carboxylase deficiency. *Lancet* 2:397
54. Gehrig, D., Leuthardt, F. 1976. A biotin-binding glycoprotein from human plasma: isolation and characterization. *10th Int. Congr. Biochem., Hamburg,* p. 209. Frankfurt: Brönners Druckerei Breidenstein
55. Ghneim, H. K., Bartlett, K. 1982. Mechanism of biotin-responsive combined carboxylase deficiency. Lancet 1:1187–88
56. Gompertz, D., Bartlett, K., Blair, D., Stern, C. M. M. 1973. Child with a defect in leucine metabolism associated with β-hydroxyisovaleric aciduria and β-methylcrotonylglycinuria. *Arch. Dis. Child.* 48:975–77
57. Gompertz, D., Draffan, G. H. 1972. The identification of tiglylglycine in the urine of a child with β-methylcrotonylglycinuria. *Clin. Chim. Acta* 37:405–10
58. Gompertz, D., Draffan, G. H., Watts, J. L., Hull, D. 1971. Biotin-responsive β-methylcrotonylglycinuria. *Lancet* 2: 22–23
59. Gompertz, D., Goodey, P. A., Bartlett, K. 1973. Evidence for the enzymatic defect in β-methylcrotonylglycinuria. *FEBS Lett.* 32:13–14
60. Green, N. M. 1963. Avidin. The use of

[^{14}C]biotin for kinetic studies and for assay. *Biochem. J.* 89:585–91
61. Grundy, W. E., Freed, M., Johnson, H. C., Henderson, C. R., Berryman, G. H. 1947. The effect of phthalylsulfathiazole (Sulfathalidine) on the excretion of B-vitamins by normal adults. *Arch. Biochem.* 15:187–94
62. György, P. 1937. Attempts to isolate the anti-egg injury factor (Vitamin H). *J. Biol. Chem.* 119:xliii-xliv
63. György, P. 1939. The curative factor (Vitamin H) for egg white injury, with particular reference to its presence in different foodstuffs and in yeast. *J. Biol. Chem.* 131:733–44
64. György, P., Rose, C. S. 1943. The liberation of biotin from the avidin-biotin complex (AB). *Soc. Exp. Biol. Med. Proc.* 53:55–57
65. György, P., Rose, C. S., Eakin, R. E., Snell, E. E., Williams, R. J. 1941. Egg-white injury as the result of nonabsorption or inactivation of biotin. *Science* 93:477–78
66. György, P., Rose, C. S., Hofmann, K., Melville, D. B., DU Vigneaud, V. 1940. A further note on the identity of vitamin H with biotin. *Science* 92:609
67. Hamil, B. M., Coryell, M., Roderuck, C., Kaucher, M., Moyer, E. Z., et al. 1947. Thiamine, riboflavin, nicotinic acid, pathothenic acid and biotin in the urine of newborn infants. *Am. J. Dis. Child.* 74:434–46
68. Hardinge, M. G., Crooks, H. 1961. Lesser known vitamins in foods. *J. Am. Diet. Assoc.* 38:240–45
69. Harris, S. A., Wolf, D. E., Mozingo, R., Folkers, K. 1943. Synthetic biotin. *Science* 97:447–48
70. Heard, G. S., McVoy, J. R. S., Wolf, B. 1984. A screening method for biotinidase deficiency in newborns. *Clin. Chem.* 30:125–27
71. Hillman, R. E., Keating, J. P., Williams, J. C. 1978. Biotin-responsive propionic acidemia presenting as the rumination syndrome. *J. Pediatr.* 92:439–41
72. Hood, R. L., Johnson, A. R. 1980. Supplementation of infant forumlations with biotin. *Nutr. Rep. Int.* 21:727–31
73. Hoppner, K., Lampi, B., Smith, D. C. 1978. An appraisal of the daily intakes of vitamin B12, pantothenic acid and biotin from a composite Canadian diet. *Can. Inst. Food Sci. Technol. J.* 11:71
74. Innis, S. M., Allardyce, D. B. 1983. Possible biotin deficiency in adults receiving long-term total parenteral nutrition. *Am. J. Clin. Nutr.* 37:185–87
75. Izumi, Y., Ogata, K. 1977. Some aspects of the microbial production of biotin. *Adv. Appl. Microbiol.* 22:145–76
76. Jakobs, C., Sweetman, L., Nyhan, W. L., Packman, S. 1984. Stable isotope dilution analysis of 3-hydroxyisovaleric acid in amniotic fluid: Contribution to the prenatal diagnosis of inherited disorders of leucine catabolism. *J. Inherit. Metab. Dis.* 7:15–20
77. Johnson, A. R., Hood, R. L., Cullen, J. A., Emery, J. L. 1978. Biotin and the sudden infant death syndrome (cot death). *Proc. Nutr. Soc. Aust.* 3:101
78. Johnson, A. R., Hood, R. L., Emery, J. L. 1980. Biotin and the sudden infant death syndrome. *Nature* 285:159–60
79. Kaplan, I. I. 1944. One-year observations of the treatment of cancer with avidin (egg white). *Am. J. Med. Sci.* 207: 733–43
80. Keeton, B. R., Moosa, A. 1976. Organic aciduria. Treatable cause of floppy infant syndrome. *Arch. Dis. Child.* 51:636–38
81. Kien, C. L., Kohler, E., Goodman, S. I., Berlow, S., Hong, R., et al. 1981. Biotin-responsive in vivo carboxylase deficiency in two siblings with secretory diarrhea receiving total parenteral nutrition. *J. Pediatr.* 99:546–50
82. Knappe, J., Brümmer, W., Biederbick, K. 1963. Reinigung und Eigenschaften der Biotinidase aus Schweinenieren und Lactobacillus Casei. *Biochem. Z.* 338: 599–613
83. Kögl, F., Tönnis, B. 1936. Über das Bios-Problem. Darstellung von krystallisiertem Biotin aus Eigelb. *Z. Physiol. Chem.* 242:43–73
84. Koivusalo, M., Pispa, J. 1963. Biotinidase activity in animal tissues. *Acta Physiol. Scand.* 58:13–19
85. Krause, K.-H., Berlit, P., Bonjour, J.-P. 1982. Erniedrigung des Biotins als möglicher Faktor im Wirkmechanismus von Antiepileptika. *Arch. Psychiatr. Nervenkr.* 231:141–48
86. Krause, K.-H., Berlit, P., Bonjour, J.-P. 1982. Impaired biotin status in anticonvulsant therapy. *Ann. Neurol.* 12: 485–86
87. Krause, K.-H., Bonjour, J.-P., Berlit, P., Kochen, W. 1985. Biotin status of epileptics. 1985. See Ref. 38, pp. 297–313
88. Krause, K.-H., Kochen, W., Berlit, P., Bonjour, J.-P. 1984. Excretion of organic acids associated with biotin deficiency in chronic anticonvulsant therapy. *Int. J. Vitam. Nutr. Res.* 54:217–22
89. Krueger, K. K., Peterson, W. H. 1948. Microbiological evidence for the identity of α and β-biotin. *J. Biol. Chem.* 173: 497–501

90. Le, P. T., Zielinska, B., Sweetman, L., Nyhan, W. L. 1985. Determination of biotinidase activity in human plasma using [^{14}C]biocytin as substrate. See Ref. 38, p. 434
91. Lease, J. G., Parsons, H. T. 1934. CCLXXVIII. The relationship of dermatitis in chicks to lack of vitamin B2 and to dietary egg-white. *Biochem. J.* 28:2109–15
92. Leonard, J. V., Seakins, J. W. T., Bartlett, K., Hyde, J., Wilson, J., Clayton, B. 1981. Inherited disorders of 3-methylcrotonyl-CoA carboxylation. *Arch. Dis. Child.* 56:53–59
93. Markkanen, T. 1960. Studies on the urinary excretion of thiamine, riboflavin, nicotinic acid, pantothenic acid and biotin in achlorhydria and after partial gastrectomy. *Acta Med. Scand.* 169 (Suppl. 360):1–52
94. Marsac, C., Gaudry, M., Augereau, C., Moncion, A., Saudubray, J.-M., et al. 1983. Biotin-dependent carboxylase activities in normal human and multicarboxylase deficient patient fibroblasts: relationship to the biotin content of the culture medium. *Clin. Chim. Acta* 129: 119–28
95. McAllister, H. C., Coon, M. J. 1966. Further studies on the properties of liver propionyl coenzyme A holocarboxylase synthetase and the specificity of holocarboxylase formation. *J. Biol. Chem.* 241:2855–61
96. McClain, C. J., Baker, H., Onstad, G. R. 1982. Biotin deficiency in an adult during home parenteral nutrition. *J. Am. Med. Assoc.* 247:3116–17
97. Melville, D. B., Moyer, A. W., Hofmann, K., DU Vigneaud, V. 1942. The structure of biotin: The formation of thiophenevaleric acid from biotin. *J. Biol. Chem.* 146:487–92
98. Messaritakis, J., Kattamis, C., Karabula, C., Matsaniotis, N. 1975. Generalized seborrhoeic dermatitis. Clinical and therapeutic data of 25 patients. *Arch. Dis. Child.* 50:871–74
99. Mock, D. M., Baswell, D. L., Baker, H., Holman, R. T., Sweetman, L. 1985. Biotin deficiency complicating parenteral alimentation: Diagnosis, metabolic repercussions, and treatment. See Ref. 38, pp. 314–34
100. Mock, D. M., Baswell, D. L., Baker, H., Holman, R. T., Sweetman, L. 1985. Biotin deficiency complicating parenteral alimentation: Diagnosis, metabolic repercussions, and treatment. *J. Pediatr.* 106:762–69
101. Mock, D. M., DeLorimer, A. A., Liebman, W. M., Sweetman, L., Baker, H. 1981. Biotin deficiency. An unusual complication of parenteral alimentation. *N. Engl. J. Med.* 304:820–23
102. Moss, J., Lane, M. D. 1971. The biotin-dependent enzymes. *Adv. Enzymol.* 35: 321–442
103. Munnich, A., Saudubray, J.-M., Carré, G., Coudé, F. X., Ogier, H., et al. 1981. Defective biotin absorption in multiple carboxylase deficiency. *Lancet* 2:263
104. Munnich, A., Saudubray, J.-M., Cotisson, A., Coudé, F. X., Ogier, H., et al. 1981. Biotin-dependent multiple carboxylase deficiency presenting as a congenital lactic acidosis. *Eur. J. Pediatr.* 137:203–6
105. Munnich, A., Saudubray, J.-M., Coudé, F. X., Charpentier, C., Saurat, J. H., Frezal, J. 1980. Fatty-acid-responsive alopecia in multiple carboxylase deficiency. *Lancet* 1:1080–81
106. Narisawa, K., Arai, N., Igarashi, Y., Satoh, T., Tada, K. 1982. Clinical and biochemical findings on a child with multiple biotin-responsive carboxylase deficiencies. *J. Inherit. Metab. Dis.* 5:67–68
107. National Academy of Sciences. 1980. Biotin. In *Recommended Dietary Allowances*, pp. 120–22. Washington, DC
108. Nisenson, A. 1957. Seborrheic dermatitis of infants and Leiner's disease: A biotin deficiency. *J. Pediatr.* 51:537–48
109. Ohrui, H., Emoto, S. 1975. Stereospecific synthesis of (+)-biotin. *Tetrahedron Lett.* 32:2765–66
110. Oppel, T. W. 1942. Studies of biotin metabolism in man. Part I. The excretion of biotin in human urine. *Am. J. Med. Sci.* 204:856–75
111. Oppel, T. W. 1948. Studies of biotin metabolism in man. IV. Studies of the mechanism of absorption of biotin and the effect of biotin administration on a few cases of seborrhea and other conditions. *Am. J. Med. Sci.* 215:76–83
112. Packman, S., Caswell, N. M., Baker, H. 1982. Biochemical evidence for diverse etiologies in biotin-responsive multiple carboxylase deficiency. *Biochem. Genet.* 20:17–28
113. Packman, S., Caswell, N. M., Gonzalez-Rios, M. D. C., Kadlecek, T., Cann, H., et al. 1984. Acetyl CoA carboxylase in cultured fibroblasts: Differential biotin dependence in the two types of biotin-responsive multiple carboxylase deficiency. *Am. J. Hum. Genet.* 36:80–92
114. Packman, S., Cowan, M. J., Golbus, M. S., Caswell, N. M., Sweetman, L., etal. 1982. Prenatal treatment of biotin-responsive multiple carboxylase deficiency. Lancet 1:1435–40

115. Packman, S., Sweetman, L., Baker, H., Wall, S. 1981. The neonatal form of biotin-responsive multiple carboxylase deficiency. *J. Pediatr.* 99:418–20
116. Packman, S., Sweetman, L., Yoshino, M., Baker, H., Cowan, M. 1981. Biotin-responsive multiple carboxylase deficiency of infantile onset. *J. Pediatr.* 99:421–23
117. Parsons, H. T. 1931. The physiological effects of diets rich in egg white. *J. Biol. Chem.* 90:351–67
118. Parsons, H. T., Kelly, E. 1933. The effect of heating egg white on certain characteristic pellagra-like manifestations produced in rats by its dietary use. *Am. J. Physiol.* 104:150–64
119. Pearce, J., Balnave, D. 1978. A review of biotin deficiency and the fatty liver and kidney syndrome in poultry. *Br. Vet. J.* 134:598–609
120. Petrelli, F., Moretti, P., Campanati, G. 1981. Studies on the relationships between biotin and the behaviour of B and T lymphocytes in the guinea-pig. *Experientia* 37:1204–6
121. Rasmussen, K., Ando, T., Nyhan, W. L., Hull, D., Cottom, D., et al. 1972. Excretion of tiglylglycine in propionic acidemia. *J. Pediatr.* 81:970–72
122. Rhoads, C. P., Abels, J. C. 1943. The administration of egg white and avidin concentrates to patients with cancer. *J. Am. Med. Assoc.* 121:1261–63
123. Robinson, B. H., Oei, J., Saunders, M., Gravel, R. 1983. [^{3}H]Biotin-labeled proteins in cultured human skin fibroblasts from patients with pyruvate carboxylase deficiency. *J. Biol. Chem.* 258:6660–64
124. Rocco, M. D., Superti-Furga, A., Caprino, D., Oddino, N. 1984. Letter: Phenotypic variability in biotinidase deficiency. *J. Pediatr.* 104:964–65
125. Roth, K. S., Allan, L., Yang, W., Foreman, J. W., Dakshinamurti, K. 1981. Serum and urinary biotin levels during treatment of holocarboxylase synthetase deficiency. *Clin. Chim. Acta* 109:337–40
126. Roth, K., Cohn, R., Yandrasitz, J., Preti, G., Dodd, P., Segal, S. 1976. Beta-methylcrotonic aciduria associated with lactic acidosis. *J. Pediatr.* 88:229–35
127. Roth, K. S., Yang, W., Allan, L., Saunders, M., Gravel, R. A., Dakshinamurti, K. 1982. Prenatal administration of biotin in biotin responsive multiple carboxylase deficiency. *Pediatr. Res.* 16:126–29
128. Roth, K. S., Yang, W., Foreman, J. W., Rothman, R., Segal, S. 1980. Holocarboxylase synthetase deficiency: A biotin-responsive organic acidemia. *J. Pediatr.* 96:845–49
129. Sander, J. E., Packman, S., Townsend, J. J. 1982. Brain pyruvate carboxylase and the pathophysiology of biotin-dependent diseases. *Neurology* 32:878–80
130. Sarett, H. P. 1952. Effect of oral administration of streptomycin on urinary excretion of B vitamins in man. *J. Nutr.* 47:275–87
131. Saunders, M. E., Sherwood, W. G., Duthie, M., Surh, L., Gravel, R. A. 1982. Evidence for a defect of holocarboxylase synthetase activity in cultured lymphoblasts from a patient with biotin-responsive multiple carboxylase deficiency. *Am. J. Hum. Genet.* 34:590–601
132. Saunders, M., Sweetman, L., Robinson, B., Roth, K., Cohn, R., Gravel, R. A. 1979. Biotin-responsive organicaciduria. Multiple carboxylase defects and complementation studies with propionic acidemia in cultured fibroblasts. *J. Clin. Invest.* 64:1695–1702
133. Scott, D. 1958. Clinical biotin deficiency (egg white injury). *Acta Med. Scand.* 162:69–70
134. Secor McVoy, J. R., Heard, G. S., Wolf, B. 1984. Potential for prenatal diagnosis of biotinidase deficiency. *Prenatal Diagn.* 4:317–18
135. Sherwood, W. G., Saunders, M., Robinson, B. H., Brewster, T., Gravel, R. A. 1982. Lactic acidosis in biotin-responsive multiple carboxylase deficiency caused by holocarboxylase synthetase deficiency of early and late onset. *J. Pediatr.* 101:546–50
136. Sorrell, M. F., Frank, O., Thomson, A. D., Aquino, H., Baker, H. 1971. Absorption of vitamins from the large intestine *in vivo Nutr. Rep. Int.* 3:143–48
137. Spencer, R. P., Brody, K. R. 1964. Biotin transport by small intestine of rat, hamster, and other species. *Am. J. Physiol.* 206:653–57
138. Stokke, O., Eldjam, L., Jellum, E., Pande, H., Waaler, P. E. 1972. Beta-methylcrotonyl-CoA carboxylase deficiency: A new metabolic error in leucine degradation. *Pediatrics* 49:726–35
139. Suchy, S. F., Wolf, B. 1982. Protein-bound biotin: A consideration in multiple carboxylase deficiency. *Lancet* 1: 108
140. Suormala, T., Wick, H., Bonjour, J.-P., Baumgartner, E. R. 1985. Rapid differential diagnosis of carboxylase deficiencies and evaluation for biotin-responsiveness in a single blood sample. *Clin. Chim. Acta* 145:151–62
141. Švejcar, J., Homolka, J. 1950. Ex-

perimental experiences with biotin in babies. *Ann. Paediatr.* 174:175–93
142. Sweetman, L. 1981. Two forms of biotin-responsive multiple carboxylase deficiency. *J. Inherit. Metab. Dis.* 4:53–54
143. Sweetman, L., Bates, S. P., Hull, D., Nyhan, W. L. 1977. Propionyl-CoA carboxylase deficiency in a patient with biotin-responsive 3-methylcrotonylglycinuria. *Pediatr. Res.* 11:1144–47
144. Sweetman, L., Nyhan, W. L., Sakati, N. A., Ohlsson, A., Mange, M. S., et al. 1982. Organic aciduria in neonatal multiple carboxylase deficiency. *J. Inherit. Metab. Dis.* 5:49–53
145. Sweetman, L., Surh, L., Baker, H., Peterson, R. M., Nyhan, W. L. 1981. Clinical and metabolic abnormalities in a boy with dietary deficiency of biotin. *Pediatrics* 68:553–58
146. Swick, H. M., Kien, C. L. 1983. Biotin deficiency with neurologic and cutaneous manifestations but without organic aciduria. *J. Pediatr.* 103:265–67
147. Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N. M., Isbell, H. 1942. Observations on the "egg white injury" in man. *J. Am. Med. Assoc.* 118:1199–1200
148. Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N. M., Isbell, H. 1942. Preliminary observations on "egg white injury" in man and its cure with a biotin concentrate. *Science* 95: 176–77
149. Taitz, L. S., Green, A., Strachan, I., Bartlett, K., Bennet, M. 1983. Biotinidase deficiency and the eye and ear. *Lancet* 2:918
150. Thoene, J., Baker, H., Yoshino, M., Sweetman, L. 1981. Biotin-responsive carboxylase deficiency associated with subnormal plasma and urinary biotin. *N. Engl. J. Med.* 304:817–20
151. Thoene, J. G., Lemons, R., Baker, H. 1983. Impaired intestinal absorption of biotin in juvenile multiple carboxylase deficiency. *N. Engl. J. Med.* 308:639–42
152. Thoene, J., Wolf, B. 1983. Biotinidase deficiency in juvenile multiple carboxylase deficiency. *Lancet* 2:398
153. Thoma, R. W., Peterson, W. H. 1954. The enzymatic degradation of soluble bound biotin. *J. Biol. Chem.* 210:569–79
154. Thompson, R. C., Eakin, R. E., Williams, R. J. 1941. The extraction of biotin from tissues. *Science* 94:589–90
155. Traub, W. 1956. Crystal structure of biotin. *Nature* 178:649–50
156. Turner, J. B., Hughes, D. E. 1962. The absorption of some B-group vitamins by surviving rat intestine preparations. *Q. J. Exp. Physiol.* 47:107–23
157. Vallotton, M., Hess-Sander, U., Leuthardt, F. 1965. Fixation spontanée de la biotine à une protéine dans le sérum humain. *Helv. Chim. Acta* 48:126–33
158. Wastell, H., Dale, G., Bartlett, K. 1984. A sensitive fluorimetric rate assay for biotinidase using a new derivative of biotin, biotinyl-6-aminoquinoline. *Anal. Biochem.* 140:69–73
159. West, P. M., Wilson, P. W. 1939. The relation of "coenzyme R" to biotin. *Science* 89:607–8
160. Weyler, W., Sweetman, L., Maggio, D. C., Nyhan, W. L. 1977. Deficiency of propionyl-CoA carboxylase and methylcrotonyl-CoA carboxylase in a patient with methylcrotonylglycinuria. *Clin. Chim. Acta* 76:321–28
161. Williams, M. L., Packman, S., Cowan, M. J. 1983. Alopecia and periorificial dermatitis in biotin-responsive multiple carboxylase deficiency. *J. Am. Acad. Dermatol.* 9:97–103
162. Williams, R. H. 1943. Clinical biotin deficiency. *N. Engl. J. Med.* 228:247–52
163. Wilson, J., Lorenz, K. 1979. Biotin and choline in foods—Nutritional importance and methods of analysis: A review. *Food Chem.* 4:115–29
164. Wolf, B., Grier, R. E., Allen, R. J., Goodman, S. I., Kien, C. L. 1983. Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. *Clin. Chim. Acta* 131:273–81
165. Wolf, B., Grier, R. E., Allen, R. J., Goodman, S. I., Kien, C. L., et al. 1983. Phenotypic variation in biotinidase deficiency. *J. Pediatr.* 103:233–37
166. Wolf, B., Grier, R. E., Heard, G. S. 1983. Hearing loss in biotinidase deficiency. *Lancet* 2:1365–66
167. Wolf, B., Grier, R. E., Parker, W. D., Goodman, S. I., Allen, R. J. 1983. Deficient biotinidase activity in late-onset multiple carboxylase deficiency. *N. Engl. J. Med.* 308:161
168. Wolf, B., Grier, R. E., Secor McVoy, J. R., Heard, G. S. 1985. Biotinidase deficiency: A novel vitamin recycling defect. *J. Inherit. Metab. Dis.* 8(Suppl. 1):53–58
169. Wolf, B., Heard, G. S., Jefferson, L. G., Proud, V. K., Nance, W. E., Weissbecker, K. A. 1985. Clinical findings in four children with biotinidase deficiency detected through a statewide neonatal screening program. *N. Engl. J. Med.* 313:16–19
170. Wolf, B., Heard, G. S., McVoy, J. R., Raetz, H. M. 1984. Biotinidase deficiency: The possible role of biotinidase

in the processing of dietary protein-bound biotin. *J. Inherit. Metab. Dis.* 7(Suppl. 2):121–22

171. Wolf, B., Hsia, Y. E., Sweetman, L., Feldman, G., Boychuk, R. B., et al. 1981. Multiple carboxylase deficiency: Clinical and biochemical improvement following neonatal biotin treatment. *Pediatrics* 68:113–18
172. Wolf, B., Hsia, Y. E., Sweetman, L., Gravel, R., Harris, D. J., Nyhan, W. L. 1981. Propionic acidemia: A clinical update. *J. Pediatr.* 99:835–46
173. Wolf, B., Secor McVoy, J. 1983. A sensitive radioassay for biotinidase activity: deficient activity in tissues of serum biotinidase-deficient individuals. *Clin. Chim. Acta* 135:275–81
174. Wood, H. G., Barden, R. E. 1977. Biotin enzymes. *Ann. Rev. Biochem.* 46:385–413
175. Wright, L. D., Driscoll, C. A., Boger, W. P. 1954. Biocytinase, an enzyme concerned with hydrolytic cleavage of biocytin. *Proc. Soc. Exp. Biol. Med.* 86:335–37
176. Yatzidis, H., Koutsicos, D., Alaveras, A. G., Papastephanidis, C., Frangos-Plemenos, M. 1981. Biotin for neurologic disorders of uremia. *N. Engl. J. Med.* 305:764