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# REGULATION OF BIOTIN ENZYMES

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## INTRODUCTION

The discovery of biotin and the eventual elucidation of its structure and role in metabolism involved diverse investigations spanning many decades. Kogl & Tonnis (80) isolated crystalline biotin from egg yolk. Subsequently Du Vigneaud et al (34) determined the chemical structure. Harris et al (61) chemically synthesized biotin from cysteine and chloroacetic acid. Biotin was shown to be a (+)-*cis*-hexahydro-2-keto-1,H-thieno-(3,4)-imidazole-4-valeric acid. Only the (+)-stereoisomer of biotin exhibits significant biological activity.



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The molecular weight of biotin is 244.3 g/mol and it is soluble in water (0.02% w/v), ethanol (0.08% w/v), and dilute alkali. The reaction sequence for the synthesis of biotin by *E. coli* was reported by Eisenberg (37).

Various aspects of the function of biotin (104), biotin enzymes (106, 165), acetyl-CoA carboxylase (2, 22, 91), and pyruvate carboxylase (131, 144) have been reviewed. The proceedings of an international conference on biotin have been published (29). Inheritable biotin-treatable disorders were reviewed by Sweetman & Nyhan (140) in this series. The present review is primarily devoted to a discussion of the recent advances in the dietary and hormonal regulation of biotin enzymes. In view of the significant progress in the clinical management of the multiple carboxylase deficiency syndrome, a discussion of the enzymes of biotin metabolism is also included.



## BIOTIN DEFICIENCY AND BIOTIN REQUIREMENT

Spontaneous deficiency of biotin in humans is quite rare, perhaps because the vitamin is so widespread in foodstuff (141). Biotin deficiency in humans has, until recently, only been observed after prolonged ingestion of raw egg white (18). However, marginal biotin deficiency has been observed in various clinical cases. Mock et al (105) reported three patients who acquired biotin deficiency during parenteral alimentation rather than as an inborn error of metabolism such as multiple carboxylase deficiency. Other direct evidence for the existence of biotin deficiency comes from the study of epileptic patients on long-term therapy with anticonvulsants (83). Patients on primidone, phenytoin, carbamazepine, or phenobarbital, but not those on valproic acid, excrete increased amounts of organic acids, as has been observed in carboxylase deficiency. They have significantly low levels of circulating biotin. Anticonvulsants having a carbamide group may be competing with biotin for a biotin-binding protein in circulation. An analogous situation has been observed with patients on long-term hemodialysis. Yatsidis et al (168) studied nine patients undergoing chronic hemodialysis for 2–10 years and suffering from dialysis dementia and peripheral neuropathy. Addition of 10 mg of biotin per day, given in three doses, to the diet markedly improved clinical symptoms in all patients within three months of treatment.

Plants and certain bacteria can synthesize biotin and represent the ultimate source for humans. Earlier reports indicated that various cell lines do not require biotin for growth of cells. The high content of biotin in bovine serum used to culture cells might have masked the biotin requirement of these cells. Recent studies with the biotin-depleted serum demonstrated the absolute requirement of biotin for the growth of various cell lines (30). The quantitative dietary requirement for biotin in humans is not known with any degree of certainty. The daily intakes of biotin in typical West European and North

American diets have been calculated to be 50–100 and 150–300 μg respectively. A recommended dietary allowance for biotin has not been established. However, 50 μg/1000 kcal of dietary energy is expected to provide an adequate intake of biotin (111a).

There have been conflicting reports about the intestinal absorption of biotin in different species (14, 136). Various studies (21, 125) indicate that the transport of biotin was higher in the jejunum than in the ileum and was minimal in the colon. Along with the earlier observation (133) that biotin was better absorbed when given orally than when instilled into the colon, this would suggest that although biotin is synthesized by colonic microflora it might not be a significant source of biotin nutrition for the host. The biphasic transport of biotin and biocytin in the rat small intestine reported by us (31) suggests that, at the expected low concentration of biotin in the gut, the saturable mechanism would operate. If such a system indeed operated in humans it would have tremendous advantage in the context of fluctuating amounts of biotin ingested in the diet. This is borne out by the rarity of primary biotin deficiency in humans.



## BIOTIN ENZYMES

The essential requirement for biotin by the higher organisms arises from its obligatory involvement in carbohydrate and lipid metabolism and in the further utilization of the deaminated residues of certain amino acids. There are only four biotin enzymes in mammalian tissues—acetyl-CoA carboxylase, pyruvate carboxylase, propionyl-CoA carboxylase, and β-methylcrotonyl-CoA carboxylase. Each of the biotin-dependent carboxylases catalyzes an ATP-dependent $CO_2$ fixation reaction, and biotin functions as a $CO_2$ carrier on the surface of the enzymes. Acetyl-CoA carboxylase is a cytosolic enzyme whereas the other three are mitochondrial enzymes. Biotin is covalently linked to the ε-amino group of lysine in all carboxylase. The amino acid sequence near the biotin of pyruvate carboxylase from sheep, chicken, and turkey livers (124), from transcarboxylase of *Propionibacterium shermanii* (101), and from acetyl-CoA carboxylase of *E. coli* (138) shows a great deal of homology in this region. In all cases, an Ala-Met-Bct-Met (Bct is the abbreviation for biocytin) sequence occurs, and in the pyruvate carboxylase and transcarboxylase the identity extends to Ala-Met-Bct-Met-Glu-Thr. It has been suggested that this conservation provides evidence that these biotin carboxylases and transcarboxylase may have evolved from a common ancestor (165). Furthermore, the sequence may be involved in activating the biotin and/or orientating it so that it is an effective carboxyl carrier between the substrate sites. The sequence may also be important in designating the

specific lysine of the protein that is to be biotinated posttranslationally by the apoenzyme synthetase (167).

Acetyl-CoA carboxylase (EC 6.4.1.2) catalyzes the ATP-dependent carboxylation of acetyl-CoA, which leads to the formation of malonyl-CoA and is recognized to be a regulatory enzyme of lipogenesis. Homogeneous acetyl-CoA carboxylase has been purified from bovine adipose tissue (108), rat liver (70), rat mammary gland (1), rabbit mammary gland (60), chicken liver (10), porcine perirenal adipose tissue (16), and rat adipose tissue (119). All sources yield an enzyme preparation with a protomeric molecular weight between 400 and 500 kilodaltons (kD). Earlier studies indicated that acetyl-CoA carboxylase was composed of two different polypeptides. However, recently developed fast purification procedures using polyethyleneglycol precipitation and affinity chromatography on avidin-sepharose column indicates that it contains two identical subunits of between 220 and 260 kD. The enzyme contains one mole of biotin per mole of subunit as the prosthetic group. The pure enzyme has been shown to exist in an equilibrium between the inactive protomeric form and a high-molecular-weight (4 to 10 million daltons) active polymeric form (107).

Pyruvate carboxylase (EC 6.4.1.1) is a key regulatory enzyme of gluconeogenesis in liver and kidney, where it catalyzes the first step in the synthesis of glucose from pyruvate. It is also present in lipogenic tissues (liver, adipose, lactating mammary gland, and adrenals) and participates in fatty acid synthesis by transporting acetyl groups, as citrate, and reducing groups, as malate from the mitochondria to cytosol (6). In all tissues it has an anapleurotic role in the formation of oxaloacetate. Pyruvate carboxylase is present solely in the mitochondrial matrix (147) and catalyzes the formation of oxaloacetate from pyruvate and $HCO_3^-$ and utilizes ATP (145). It is a homopolymer of identical subunits, with a subunit $M_r$ of about 125,000 (7).

Propionyl-CoA carboxylase (EC 6.4.1.3) is a key enzyme in the catabolic pathway of odd-chain fatty acids, isoleucine, threonine, methionine, and valine (122). The enzyme catalyzes the conversion of propionyl-CoA to methylmalonyl-CoA, which in turn enters the tricarboxylic acid via succinyl-CoA. The native enzyme is composed of two nonidentical subunits—an $\alpha$ subunit ($M_r$ = 70,000–72,000) and a $\beta$ subunit ($M_r$ = 54,000–56,000) (52, 71). The $\alpha$ subunit contains the biotin ligand. The human enzyme has a sedimentation coefficient of 17.4S and a molecular weight of 540,000, which indicates an $\alpha_4\beta_4$ structure. This is in contrast to the sheep enzyme found to be 730–840 kD with a proposed structure of $\alpha_6\beta_6$ (48). The enzyme is found in the mitochondrial matrix. The rat $\beta$ chain has been shown to be synthesized as a precursor some 7500 daltons larger than the mature polypeptide (85).

$\beta$-Methylcrotonyl-CoA carboxylase (EC 6.4.1.4) catalyzes the conversion

of β-methylcrotonyl-CoA to β-methylglutaconyl-CoA, a key reaction in the degradative pathway of leucine. This enzyme has been purified from *Achromobacter* and shown to have a molecular weight of 760,000 (3, 64). It contains four biotins per molecule. This enzyme contains two different polypeptides of molecular weight 78,000 and 96,000 respectively and biotin is bound to the larger peptide (128).

Of the four mammalian biotin enzymes only two, acetyl-CoA carboxylase and pyruvate carboxylase, have regulatory features. Short- and long-term modulations of both enzyme activity and the amount of the enzyme protein by dietary factors and hormonal status are known to occur for these enzymes. Apart from the effects of dietary deficiency and genetic factors altering individual or all carboxylases, there is no report of changes in the activities of propionyl-CoA carboxylase and β-methylcrotonyl-CoA carboxylase. Neither of these enzymes are rate limiting in the pathways with which they are associated.



## REGULATION OF ACETYL-CoA CARBOXYLASE

Liver, adipose tissue, and the lactating mammary gland are the major sites of lipogenesis in mammalian species. It is well known that the rate of lipid synthesis is decreased during starvation and while on a fat-rich diet. Lipogenesis is markedly enhanced during refeeding of a fat-free, carbohydrate-rich diet to starved animals. Katsurada et al (72) compared the effects of individual dietary nutrients on substrate and effector levels as well as on fatty acid synthesis from tritiated water and the activities of lipogenic enzymes. They found that fatty acid synthesis corresponded more closely to acetyl-CoA carboxylase activity than to other lipogenic enzyme activities and concluded that under conditions of dietary nutrient manipulation acetyl-CoA carboxylase would be the rate-limiting enzyme of fatty acid synthesis. Hormonal, developmental, and genetic conditions that alter lipogenesis also affect this rate-limiting step in the fatty acid synthetic pathway.

### *Long-Term Regulation*

The concentration of acetyl-CoA carboxylase in the liver is highly dependent on the type of dietary energy consumed. However, the mechanism by which specific dietary constituents regulate the level of acetyl-CoA carboxylase is difficult to ascertain by nutritional studies because the direct action of a nutrient as an inducer or inhibitor of enzyme synthesis cannot be differentiated from an indirect effect caused by dietary manipulations on hormonal levels. Long-term regulation of acetyl-CoA carboxylase involves changes in the amount of the carboxylase through modification of the rates of synthesis and/or degradation of the enzyme protein (100, 110). Majerus &

Kilburn (100) showed that the rate of synthesis of rat liver acetyl-CoA carboxylase was stimulated 5- to 10-fold when previously fasted rats were fed a fat-free diet. When fasted rats were given actinomycin D prior to refeeding, there was no rise in fatty acid synthetic activity upon refeeding, which indicates that protein synthesis is required to produce the increase in acetyl-CoA carboxylase activity. Immunochemical studies suggest that the variations in acetyl-CoA carboxylase activity in liver cytosol from rats under different dietary conditions are accompanied by proportionate changes in the immunoprecipitable, enzymatically active protein (110).

Nakanishi & Numa (110) provided evidence that the relative rate of synthesis of acetyl-CoA carboxylase in rat liver was decreased 1.9-fold and 1.7-fold by fasting and diabetes respectively, while the rate of degradation was essentially the same in normal and diabetic rats. Analogous studies revealed that the elevated content of acetyl-CoA carboxylase in genetically obese mice can be attributed principally to changes in the rate of synthesis of the enzyme (111). The adipocytes of female rats are more insulin sensitive than those from male rats. Fatty acid synthesis and, presumably, the key lipogenic enzymes are significantly higher in both inguinal and retroperitoneal depots of the female as compared to the male rat (57). Flint et al (40) suggested that progesterone may be implicated in insulin binding capacity of rat adipocytes.

Dietary fructose is as active as glucose in increasing hepatic production of fatty acids in spite of its weak action on insulin secretion (170). Dietary fructose, but not glucose, stimulates lipogenesis in the diabetic rat. Thus, there is no insulin requirement for this enzyme induction. A number of lipogenic enzymes, including acetyl-CoA carboxylase, are induced in the liver of diabetic rats following fructose or glycerol feeding. This might result from the buildup of intermediates of carbohydrate metabolism (135). It is possible that cGMP might affect the induction in a manner similar to how it affects glucokinase induction (134). The biotin status of the animal also seems to play a role in the synthesis of acetyl-CoA carboxylase. In the adipose tissue of the biotin-deficient rat there is a significant accumulation of the apoenzyme of acetyl-CoA carboxylase (32) that cannot be ascribed to a decrease in the rate of degradation of the enzyme.

The complex physiological interactions imposed on cells in vivo make it difficult to assess the direct role of hormones in the long-term regulation of fatty acid synthesis. Cell cultures provide a useful system for studying the regulation of fatty acid synthesis under defined conditions. Studies carried out with cultured hepatocytes (JTC-25 P3) indicated that the addition of fatty acids to the culture medium caused a decrease in the rate of synthesis of acetyl-CoA carboxylase and had no effect on its rate of degradation (77). Acetyl-CoA carboxylase was stimulated two-fold when rat hepatocyte cultures were exposed to insulin (0.1 $\mu$M), and this insulin-dependent induction

of acetyl-CoA carboxylase activity was prevented by the addition of α-amanitin or cordycepin, which suggests a transcriptional regulation of the enzyme level (44, 74). The synthesis of acetyl-CoA carboxylase in human skin fibroblasts (132) and chicken liver cells (39) grown in lipid-free medium was stimulated by insulin. It has been shown in isolated adipocytes that insulin and epidermal growth factor stimulate lipogenesis and acetyl-CoA carboxylase activity (62). In primary cultures of rat hepatocytes, dexamethasone had a permissive effect on insulin induction of acetyl-CoA carboxylase (126). The dependence on glucocorticoid for the insulin induction of acetyl-CoA carboxylase is similar to the permissive effect of glucocorticoids in the insulin induction of glucokinase (129).

Results of our investigation with HeLa cells indicate that when the cells are cultured in a medium containing lipid-free fetal bovine serum or in Waymouth's serum-free medium acetyl-CoA carboxylase activity is increased over that of cells cultured in medium containing fetal bovine serum. This increase was associated with a corresponding increase in the relative synthesis of acetyl-CoA carboxylase. Addition of glucagon to the HeLa cell culture medium caused a 50% decrease in acetyl-CoA carboxylase activity. This was accompanied by a concordant decrease in the relative synthesis of acetyl-CoA carboxylase as measured by immunochemical techniques (15). Klandorf et al (78) pointed out that during starvation the plasma concentration of glucagon is significantly higher in biotin-deficient than in biotin-supplemented chickens. Plasma insulin was equally suppressed in both groups of birds. It is not known whether this observation is confined to the avian species. Also, the role of biotin in the secretion of glucagon has not been elucidated.

Thus, changes in amount of acetyl-CoA carboxylase caused by dietary, nutrient, and hormonal manipulations have been ascribed to alterations of enzyme synthetic rates rather than degradation (73). Similar regulation of malic enzyme of chick liver cells in culture under the influence of insulin, triiodothyronine, and glucagon was also correlated with alterations in the relative rate of synthesis of the enzyme, while the rate of degradation remained unaffected (49).

## *Short-Term Regulation*

Apart from the regulation of the amount of the enzyme protein, the activity of acetyl-CoA carboxylase is regulated by a variety of other factors, for example the functional levels of substrate, effectors, and the phosphorylation state of the enzyme. There is much contention regarding the relative physiological significance of each of these and the mechanism of action of various hormones that brings about short-term changes in the activity of acetyl-CoA carboxylase.

The activity of acetyl-CoA carboxylase is greatly increased in vitro by the

addition of citrate. The work of Beaty & Lane (11, 12) showed that citrate induces a conformational change in the enzyme leading to its activation and that dimerization of the active enzyme is the second and rate-limiting step in the polymerization sequence. Carboxylation of the biotinyl group of the polymerized enzyme leads to depolymerization. Hence the competition between citrate and malonyl-CoA in regard to the protomer-polymer equilibrium of acetyl-CoA carboxylase. There is debate about whether the cytosolic concentration of citrate is high enough to activate the enzyme and also about the concordance of citrate levels with the activity state of the enzyme (22, 76).

Acetyl-CoA carboxylase is inhibited by long-chain fatty acyl-CoA. This results in enzyme depolymerization (115). The $K_i$ for palmityl-CoA is about 5 nM, which is far lower than its critical micellar concentration. This suggests that regulation of enzyme activity by long-chain acyl-CoA might be physiologically relevant. This again has been questioned on the basis that the whole-cell concentration of long-chain acyl-CoA in the the range 50–150 $\mu$M is several orders of magnitude greater than required for in vitro inhibition of acetyl-CoA carboxylase activity (169). However, Carlson & Kim (24) reported that the dephosphorylated acetyl-CoA carboxylase requires only one tenth as much citrate for maximal enzyme activity and is more resistant to inhibition by fatty acyl-CoA. Thus, allosteric control of the enzyme is possible by metabolites at their physiological concentration ranges. Another major regulator of acetyl-CoA carboxylase activity is the coenzyme A activation of acetyl-CoA carboxylase, which lowers the $K_m$ for acetyl-CoA (169).

## *Regulation by Covalent Modification*

Since the early report of Carlson & Kim (23), inactivation of acetyl-CoA carboxylase by phosphorylation has been actively examined in various laboratories. Hormones involved in cAMP generation as well as insulin have been examined for their role in the short-term control of acetyl-CoA carboxylase. Witters et al (154) indicated that cAMP-dependent phosphorylation played a major role in regulating acetyl-CoA carboxylase activity in rat liver and adipose tissue. More recently, protein phosphatase isolated from rat liver (68) was shown to dephosphorylate and activate acetyl-CoA carboxylase from rat and rabbit mammary gland and rat liver (69). Evidence that hormones such as epinephrine inhibit acetyl-CoA carboxylase via a direct phosphorylation mechanism has been presented (109). However, insulin and epidermal growth factor also cause an increase in acetyl-CoA carboxylase activity in hepatocytes and adipocytes in conjunction with increased phosphorylation (62). The principal sites phosphorylated in adipocytes exposed to insulin were quite distinct from those exposed to epinephrine (65). However, Bothwick et al (19) report that the major changes in phosphopeptide profiles in rat epididymal adipose tissue, previously exposed either to insulin or the $\beta$-adrenergic

agonist isoproterenol, represent changes in phosphorylation of the same phosphoprotein. Thus, the role of cAMP-dependent phosphorylation of acetyl-CoA carboxylase as the only direct regulator of this enzyme activity in all tissues is still in question. Earlier reports (46, 96) indicate that purified rat liver acetyl-CoA carboxylase could not be phosphorylated and inactivated in the presence of the catalytic subunit of cAMP-dependent protein kinase. A cAMP-independent protein kinase has been isolated from rat liver. This is CoA dependent, and it phosphorylates and inactivates acetyl-CoA carboxylase (82). We reported a similar regulation of porcine adipose tissue acetyl-CoA carboxylase via phosphorylation by a cAMP-independent and CoA-dependent protein kinase (16).

Hardie et al (59) have pointed out that insulin and epidermal growth factor bring about the breakdown of phosphatidylinositol bisphosphate in cell membrane, which then releases second messengers such as inositol trisphosphate that cause release of intracellular calcium. This could activate calmodulin-dependent multiprotein kinase, which phosphorylates acetyl-CoA carboxylase. The site phosphorylated on acetyl-CoA carboxylase is within the same tryptic peptide as the site phosphorylated in intact cells in response to insulin. The physiological significance of this phosphorylation is not known as yet.

Kim (76) suggested that adenylate energy charge alters phosphorylation and activation of acetyl-CoA carboxylase. At high levels of ATP the phosphorylation and inactivation of acetyl-CoA carboxylase catalyzed by the endogenous protein kinase are inhibited. An excess of AMP (and also cAMP) accelerates this. The potential significance of alterations in intracellular nucleotide concentration on acetyl-CoA carboxylase activity needs to be determined (153).

Phosphorylation/dephosphorylation seems to have other effects on lipogenic enzymes. Under conditions favoring lipogenesis a high-molecular-weight species of acetyl-CoA carboxylase was isolated that did not cosediment with the in vitro polymerized enzyme. ATP-citrate lyase, acetyl-CoA carboxylase, and fatty acid synthetase were all associated as a high-molecular-weight complex. Phosphorylation of the isolated complex dissociated the complex. This could be yet another factor in regulating lipogenesis (45).

## REGULATION OF PYRUVATE CARBOXYLASE

Short-term regulation of pyruvate carboxylase has been studied in detail. This does not involve changes in the rate of synthesis of enzyme protein. The enzyme is activated by $K^+$, $Mg^{2+}$, its substrate pyruvate, and positive effector acetyl-CoA (102, 103, 130, 146). It is inhibited by the product MgADP (102) and $SO_4^{-2}$ (130). Attwood & Wallace (4) have shown that

regulation by a positive effector, acetyl-CoA, involves changes in the enzyme molecule to a tight, tetrahedron-like conformation.

Long-term regulation of pyruvate carboxylase is influenced by the insulin status of animal. The enzyme activity increases in the liver of diabetic (150) and hyperthyroid (149) rats. Thyroidectomy decreases enzyme activity (149). Changes in catalytic activity observed in these states were shown to be directly related to intracellular concentration of enzyme, which in turn is regulated at the level of enzyme synthesis. It was further shown that under these conditions the rate of enzyme degradation was not altered. These observations are consistent with the view that gluconeogenesis in liver may be regulated by changes in the intracellular levels of pyruvate carboxylase (20).

The 3T3-L1 subline differentiates in the resting state into a cell line having the characteristics of mammalian adipocyte (55, 56, 123). During this process there is a large increase in cytosolic triglyceride. These changes are accompanied by parallel changes in the activities of many lipogenic enzymes, including acetyl-CoA carboxylase (99), ATP-citrate lyase (99), malic enzyme (87), fatty acid synthetase (137, 152), and glycerol-3-phosphate acyltransferase (88). It has been shown that the activity of pyruvate carboxylase increases 20–30-fold during differentiation and is due to an increase in the intracellular concentration of the enzyme (25, 42, 43, 98). Furthermore, the turnover of pyruvate carboxylase in differentiated cells was similar to that in undifferentiated cells. The turnover of the apoenzyme was not different from that of the holoenzyme. These results suggest that pyruvate carboxylase is regulated at the level of enzyme synthesis and is independent of its cofactor, biotin, as shown for alanine aminotransferase and tyrosine aminotransferase (93). Thus, induction of pyruvate carboxylase in this cell line is consistent with its role in lipogenesis.

## RECOMBINANT cDNA FOR BIOTIN ENZYMES

Recombinant cDNA clones for three biotin enzymes have been isolated from mammalian sources and are very useful in elucidating the process and factors involved in the regulation of these genes. Bai et al (5) recently isolated a 1.2-kilobase cDNA clone for acetyl-CoA carboxylase from rat mammary gland. They showed that acetyl-CoA carboxylase mRNA is about 10 kilobases (kbase) in size. This cDNA hybridizes to two main RNA species: one is 10 kbase and the other is a somewhat diffuse band of 3.0 kbase. They offer no information as to the nature of the 3.0-kbase RNA species. Using a $^{32}$P-labelled insert, it has been shown for the first time that the increased amount of acetyl-CoA carboxylase in rat liver occurring after eating a fat-free diet is mainly due to an increased amount of acetyl-CoA carboxylase mRNA (5).

This confirms the earlier notion that long-term regulation of acetyl-CoA carboxylase involves transcriptional control.

A cDNA clone 0.85 kbase in length for human pyruvate carboxylase has been isolated (41). This probe revealed that pyruvate carboxylase mRNA from human, baboon, and rat liver was 4.2 kbase pairs in length. Pyruvate carboxylase gene was localized on the long arm of human chromosome 11. It was further demonstrated in 3T3-L1 cells that pyruvate carboxylase mRNA content increased 23-fold in seven days after the onset of differentiation.

Complementation studies of fibroblasts deficient in propionyl-CoA carboxylase indicate that there is considerable range of genetic heterogeneity (53, 164). Two principal groups were identified through complementation analysis and designated pccA and pccBC respectively. They represent defects in two different structural genes (155, 161). It was shown that members of the pccA group failed to complement each other but complemented mutants of all other groups. The pccBC group has been further divided into subgroups pccB and pccC. These subgroups complemented each other in addition to pccA mutants, but they did not complement pccBC mutants (54, 162). The occurrence of two complementation groups suggested involvement of two structural genes in the expression of $\alpha$ peptide by the pccA group and $\beta$ peptide by the pccBC group. Complementary DNA clones coding for $\alpha$ and $\beta$ polypeptides of human propionyl-CoA carboxylase $\alpha$ and $\beta$ chain respectively were 2.9 and 2.0 kbase in length. Propionyl-CoA carboxylase $\alpha$- and $\beta$-chain genes have been assigned to independent chromosomes 13 and 3 respectively. This, together with the assignment of pyruvate carboxylase to chromosome 11, indicates the independent nature of the genes coding for the various biotin-dependent enzymes. Furthermore, several pccA mutants were deficient in propionyl-CoA carboxylase $\alpha$-chain mRNA but had normal $\beta$-chain mRNA (90), which strongly supports the earlier observation that pccA mutants involve the defect at pccA locus (89).

Although molecular cloning for biotin enzymes has only just started, this approach shows considerable promise for investigation of biotin enzymes. Complementary DNA clones will help in the isolation of genes (genomic DNA) for these enzymes. This will further elucidate the *trans*- and *cis*-acting elements involved in dietary, hormonal, developmental, and tissue-specific expression of biotin enzymes. Furthermore, the nature of mutations leading to carboxylase disorder will be precisely defined.

## SINGLE AND MULTIPLE CARBOXYLASE DEFICIENCY

Biotin deficiency or dependency, an entity that was once virtually ignored in clinical medicine, is attracting much interest now because of the progress

made in the study of inborn metabolic disorders (142). Since 1976, the incidence in infants of organic acidemia has been investigated with new analytical techniques. The acidemia has been attributed to a lack of one or more of the biotin carboxylases. Inherited disorders of individual biotin carboxylases have been reported and are distinct from the biotin-responsive multiple carboxylase deficiency because these patients do not respond even to pharmacological doses of biotin.

## *Single Carboxylase Deficiency*

Inherited deficiency of each of four carboxylases has been reported in humans. These deficiencies arise from the synthesis of abnormal biotin carboxylases and patients do not respond to biotin therapy.

Blom et al (17) reported an acetyl-CoA-carboxylase-deficient patient. Biochemical abnormalities observed in this patient were accumulation of 2-ethyl-3-ketohexanoyl-CoA, 2-ethyl-3-hydroxyhexanoic acid and 2-ethylhexaedioic acid. Patients with isolated $\beta$-methylcrotonyl-CoA carboxylase deficiency can be divided into two categories: a biotin-responsive group to which most of the previously published patients belong (17, 47, 75, 94, 95) and a group of biotin-resistant patients (8, 13). These patients excreted in urine large amounts of 3-methylcrotonylglycine and 3-hydroxyisovaleric acid. The clinical manifestation, treatment, and diagnosis of propionicacidemia (propionyl-CoA carboxylase deficiency) are reviewed elsewhere (162). The biochemical abnormalities are elevated concentrations of propionic acid and lactic acid in blood and elevated levels of secondary metabolites such as 3-hydroxypropionic acid, 2-methylcitrate, and propionylglycine in urine. Pyruvate carboxylase deficiency causes an elevation of lactic acid, pyruvic acid, and alanine levels in blood (33, 121).

## *Multiple Carboxylase Deficiency*

Attention has recently been focused on the condition referred to as multiple carboxylase deficiency, in which deficiencies of all three mitochondrial carboxylases are seen in the patient. Two distinct types of multiple carboxylase deficiencies have been recognized based on the age of onset as well as the nature of the clinical presentation. This disease manifests itself in either a neonatal or infantile form (caused by the deficiency of holocarboxylase synthetase) and a late-onset or juvenile form (caused by the deficiency of biotinidase) (117, 139, 156, 157). The incidence of biotinidase deficiency is estimated to be about 1 in 41,000 newborns (with 95% confidence limits of 1 in 12,000 to 1 in 240,000) (159). This places the incidence of biotinidase deficiency well within the range of that of other metabolic disorders that are currently included in many screening programs. However, no direct evidence exists for a holocarboxylase synthetase deficiency (neonatal form of multiple

carboxylase deficiency) because the assay procedures for holocarboxylase synthetase are so tedious.

## ENZYMES OF BIOTIN METABOLISM

### *Biotin Holocarboxylase Synthetase*

Biotin carboxylases are synthesized in the form of the apoproteins that undergo posttranslation covalent modification by the addition of biotin, the prosthetic group, to the $\epsilon$-amino group of lysine of the apoproteins. This covalent attachment of biotin to a specific lysine in the apoenzyme is catalyzed by biotin holocarboxylase synthetase in a two-step reaction:

$$\text{ATP} + \text{biotin} \rightarrow \text{5'-adenylate-biotin} + \text{PPi} \quad 1.$$

$$\text{5'-adenylate-biotin} + \text{apoenzyme} \rightarrow \text{holoenzyme} + \text{AMP} \quad 2.$$

Holocarboxylase synthetase is responsible for the formation of the holoform of $\beta$-methylcrotonyl-CoA carboxylase (66), acetyl-CoA carboxylase (97), propionyl-CoA carboxylase, and pyruvate carboxylase (92) from their respective apoenzymes. Holocarboxylase synthetase has recently been reviewed (51).

Eisenberg et al (38) purified this enzyme to homogeneity from *E. coli* and showed that apart from biotinating the apocarboxylases this enzyme regulates the gene(s) for biotin metabolism. They have drawn a parallel between the reactions involved in aminoacyl-tRNA synthesis and biotin holoenzyme synthesis, in the light of the regulatory role proposed for aminoacyl-tRNA (36). It was suggested that the holoenzyme synthetase when bound to biotinyl-5'-AMP functions as the co-repressor for the biotin operon. In the *trp* operon, the most extensively studied system (116), the trp-tRNA acts as a negative modulator at the attenuator site, terminating transcription of the *trp* operon under conditions of tryptophan excess or as a substrate for protein synthesis when the levels are low. In the biotin operon, the biotinyl-5'-AMP complex either inhibits initiation of transcription by binding to the operator site or utilizes the active form of the biotin complex that is common to both reactions. Biotin operon from *E. coli* has been extensively studied and has been sequenced (37, 67).

Eisenberg et al (38) have shown that *E. coli* holocarboxylase synthetase is a single polypeptide of $M_r$ = 34,000. Wood et al (166) found that the native enzyme from *Propionibacterium shermanii* had a $M_r$ of 29,000 ± 5,000. The enzyme from *Bacillus strearothermophilus* is reported to have a $M_r$ of 40,000 (92). There is very little information available on the eukaryotic holocarboxylase synthetase. Even basic information like molecular weight and intra-

cellular localization is not precisely known. Hopner & Knappe (66) reported $M_r$ = 50,000–100,000 for the animal synthetase. In experimental biotin deficiency there is continued synthesis of the various apoenzymes, and the tissues contain a mixture of apo- and holocarboxylases. The tissue distribution of the apo- and holoenzymes and the response, in terms of apo- and holoenzyme conversion following biotin administration to the biotin-deficient rat, suggest the possibility of an uneven tissue distribution of the holocarboxylase synthetase (32).

## *Biotinidase*

Acid hydrolysis of biotin proteins releases free biotin whereas proteolytic hydrolysis results in the formation of biotin peptides. The smallest among these peptides is biocytin, *N*-ε-(*d*-biotinyl)-L-lysine. Biotinidase (biotinamide aminohydrolyase, EC 3.5.1.12) releases biotin from the proteolytic degradation product of carboxylases or biocytin so that biotin can be reutilized. This enzyme has been detected in most mammalian tissues, with high activities being present in liver, kidney, serum, intestine, and adrenal glands (118). It has been localized in the microsomal fraction of hepatocytes (63, 118). This enzyme was recently purified to homogeneity from human serum (26, 28). Purified enzyme is a single polypeptide of $M_r$ = 68,000 and is a glycoprotein (26). The pI of this enzyme is 4.6 (28).

Various assays for biotinidase are available. The colorimetric determination of 4-aminobenzoate released from the synthetic substrate *N*-(*d*-biotinyl)-4-aminobenzoate has generally been used to assay biotinidase (79). Wolf & McVoy (163) developed a radiochemical assay in which the release of [$^{14}$C]carboxyl-4-aminobenzoate from *N*-(*d*-biotinyl)-[$^{14}$C]carboxyl-4-aminobenzoate was measured. A fluorometric assay measuring the release of 6-aminoquinoline has been described (148). These are all synthetic substrates. A fluorometric assay based on the release of lysine from the natural substrate biocytin has been reported (35).

Several questions about the function of biotinidase remain unanswered. First, where does the enzyme primarily function? Biotinidase has been detected in various tissues. Baumgartner et al (9) have shown that in biotinidase-deficient patients there is an increase in renal clearance of biotin; this suggests a role for biotinidase in renal absorption. Biotinyl peptides originating from degraded carboxylases are not recycled in the cells, but in the extracellular compartment (43, 63). It is suggested that serum biotinidase is a product of the active secretory function of liver (118, 151). Human serum biotinidase is a sialoglycoprotein; however, the sialyl group on the protein moiety is not required for enzyme activity. Sialylation might be a general mechanism of transport of secretory proteins from liver. Based on these results it was proposed that biotinidase is a secretory enzyme that releases biotin from the products of carboxylase degradation that reach the blood (158). We do not

know the primary site of the physiological action of biotinidase. It may be the liver, kidney, or plasma compartment.

The second question concerns the nature of the substrate utilized by biotinidase. Biotinidase does not appear to be a typical peptidase or esterase. It does not liberate the biotin from holocarboxylases (50). The natural substrate for biotinidase is probably biocytin since *(Streptomyces griseus)* protease (112, 113) releases biocytin from rat liver propionyl-CoA carboxylase (81). We have shown that [$^3$H]-biotin was not released by the action of biotinidase on [$^3$H]-biotinylated ribonuclease and polylysines ($M_r$ = 100,000–3,000) (26). Craft et al (28) studied the action of biotinidase on polypeptides of various length (number of residues = 5–123) having the carboxylase-conserved sequence around biocytin. The activity of biotinidase increased as the peptide chain length decreased but was significantly lower than for biocytin. These results suggest that biotin-containing proteins must be digested by proteases and peptidases to biocytin before the biotin can be recycled by the action of biotinidase.

Another question that needs to be examined is whether biotinidase is a binding or carrier protein for biotin in blood. Using various chromatographic techniques we have shown that, in human serum, biotinidase is the only protein that binds biotin; it does so with a $K_d$ of 50 nM (27). This high binding is analogous to other vitamin-binding proteins. For example, thiamine-binding protein binds thiamine with a $K_d$ of $5.5 \times 10^{-7}$M (112). Epileptic patients receiving a high daily dose of anticonvulsant (containing a carbamide group) have lower serum biotin levels than those receiving a low dose, which suggests that in these patients anticonvulsants possibly compete with biotin for biotin-binding protein in serum (83). We have shown in vitro with human serum and purified biotinidase that this is indeed the case (27). Our biotin-binding studies with human serum support the notion that biotinidase is a biotin carrier protein in human serum.

Finally, does biotinidase have a role in the intestinal absorption of biotin? The physiological significance of biotinidase in the intestinal uptake of biotin is speculative at present. Recent studies indicate that biotin transport in rat intestine is a carrier-mediated process at the low concentration range. At high concentrations transport is by simple diffusion (21, 125). Similarly, in hamster intestinal mucosal cells biotin is taken up via facilitated diffusion (50). As most of the biotin in foods such as meat and cereals is protein bound (58, 127, 143), enzymatic hydrolysis in the gastrointestinal tract would result in the release of biocytin (or biotinylpeptides) rather than of free biotin. High biotinidase activity has been reported in the rat stomach and small intestine (160). We have shown that the biphasic uptake of biocytin in rat intestine is similar to biotin uptake. Our results suggest that a single protein function in the cytosol and brush border is present in which biotinidase activity co-migrates with the biotin-binding activity (31). The implied role of biotini-

dase in transport of biotin will have to be confirmed using biotinidase antibody.

## CONCLUSION

The role of biotin as the prosthetic group of biotin-dependent $CO_2$ fixation enzymes is well recognized. In mammalian tissues there are only four enzymes containing biotin as the prosthetic group, namely acetyl-CoA carboxylase, propionyl-CoA carboxylase, pyruvate carboxylase, and $\beta$-methylcrotonyl-CoA carboxylase. The effects of biotin deficiency in animals, including humans, are quite severe involving various systems and might be explained on the basis of the participation of biotin in the regulation of certain proteins. Of the biotin-containing enzymes, only acetyl-CoA carboxylase and pyruvate carboxylase are regulated on both a short-term and long-term basis by dietary, nutritional, and hormonal manipulations. The molecular mechanisms of these regulations are currently being unravelled. Complementary DNA clones for three biotin enzymes were isolated and it was shown conclusively that long-term regulation of pyruvate carboxylase and acetyl-CoA carboxylase is at the mRNA level. Of the enzymes of biotin metabolism, biotinidase has been isolated. It seems to be the only protein in serum capable of binding specifically to biotin. Its role in biotin absorption from the gut and transport in the extracellular compartment are being investigated. To date, the biotin holocarboxylase synthetase has not been isolated from a nonbacterial source. We also do not as yet have reliable information on the human requirement and mode of uptake of biotin. Development in these areas would lead to a better understanding of biotin nutrition.

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### *Literature Cited*

1. Ahmad, F., Ahmad, P. M., Pieretti, L., Watters, G. T. 1978. Purification and subunit structure of rat mammary gland acetyl coenzyme A carboxylase. *J. Biol. Chem.* 253:1733–37
2. Alberts, A. W., Vagelos, P. R. 1972. Acyl-CoA carboxylase. *Enzymes* 6:37–82
3. Apitz-Castro, R., Rehn, K., Lynen, F. 1970. $\beta$-Methylcrotonyl-CoA carboxylase. Kristallisation und einige physikalische eigenschaften. *Eur. J. Biochem.* 16:71–79
4. Attwood, P. V., Wallace, J. C. 1986. The carboxylation complex of chicken liver pyruvate carboxylase. A kinetic analysis of the effects of acetyl-CoA, $Mg^{2+}$ ions and temperature on its stability and on its reaction with 2-oxobutrate. *Biochem. J.* 235:359–64
5. Bai, D. H., Pape, E., Lopex-Casilas, F., Luo, X. C., Dixon, J. E., Kim, K. 1986. Molecular cloning of cDNA for acetyl-coenzyme A carboxylase. *J. Biol. Chem.* 261:12395–99
6. Ballard, F. J., Hanson, R. W. 1967. The

citrate cleavage pathway and lipogenesis in rat adipose tissue: replenishment of oxaloacetate. *J. Lipid Res.* 8:73–79
7. Barden, R. E., Taylor, B. L., Isohashi, F., Frey, W. H. II, Zander, G., et al. 1975. Structural properties of pyruvate carboxyalse from chicken liver and other sources. *Proc. Natl. Acad. Sci. USA* 72:4308–12
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 hypoglycemia. *J. Inherited Metab. Dis.* 7:182
9. Baumgartner, E.R., Suormala, T., Wick, H., Bausch, J., Bonjour, J.-P. 1985. Biotinidase deficiency associated with renal loss of biocytin and biotin. *Ann. NY Acad. Sci.* 447:297–313
10. Beaty, N. B., Lane, M. D. 1982. Acetyl coenzyme A carboxylase. Rapid purification of the chick liver enzyme and steady state kinetic analysis of the carboxylase-catalyzed reaction. *J. Biol. Chem.* 257:924–29
11. Beaty, N. B., Lane, M. D. 1983. Kinetics of activation of acetyl-CoA carboxylase by citrate. Relationship to the rate of polymerization on the enzyme. *J. Biol. Chem.* 258:13043–50
12. Beaty, N. B., Lane, M. D. 1983. The polymerization of acetyl-CoA carboxylase. *J. Biol. Chem.* 258:13051–55
13. Beemer, F. A., Bartlett, K., Duran, M., Ghneim, H. K., Wadman, S. K., et al. 1982. Isolated biotin-resistant 3-methylcrotonyl-CoA carboxylase deficiency in two sibs. *Eur. J. Pediatr.* 138:351–54
14. Berger, E., Long, E., Semenza, G. 1972. The sodium activation of biotin absorption in hamster small intestine *in vivo*. *Biochim. Biophys. Acta* 255:873–87
15. Bhullar, R. P., Dakshinamurti, K. 1985. Regulation of acetyl-CoA carboxylase by glucagon in HeLa cells. *Biosci. Rep.* 5:491–97
16. Bhullar, R. P., Dakshinamurti, K. 1987. Regulation of porcine adipose tissue acetyl-CoA carboxylase phosphorylation-dephosphorylation. *Biochem. Life Sci. Adv. (India)* 6:205–10
17. Blom, W., De Muinck Keizer, S. M. P. F., Scholte, H. R. 1981. Acetyl-CoA carboxylase deficiency: an inborn error of de novo fatty acid synthesis. *N. Engl. J. Med.* 305:465–66
18. Bonjour, J-P. 1977. Biotin in man's nutrition and therapy—a review. *Int. J. Vitam. Nutr. Res.* 47:107–18
19. Bothwick, A. C., Edgell, N. J., Denton, R. M. 1986. Mechanism involved in the short-term regulation of acetyl-CoA carboxylase by insulin. *Biochem. Soc. Trans.* 14:563–65
20. Bottger, I., Kriegel, H., Wieland, O. 1970. Fluctuation of hepatic enzymes important in glucose metabolism in relation to thyroid function. *Eur. J. Biochem.* 13:253–57
21. Bowman, B. B., Selhub, J., Rosenberg, I. H. 1986. Intestinal absorption of biotin in the rat. *J. Nutr.* 116:1266–71
22. Brownsey, R. W., Denton, R. M. 1987. Acetyl-coenzyme A carboxylase. *Enzymes* 18:123–46
23. Carlson, C. A., Kim, K.-H. 1974. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. *Arch. Biochem. Biophys.* 164:478–89
24. Carlson, C. A., Kim, K.-H. 1974. Differential effects of metabolites on the active and inactive forms of hepatic acetyl CoA carboxylase. *Arch. Biochem. Biophys.* 164:490–501
25. Chandler, C. S., Ballard, F. J. 1983. Inhibition of pyruvate carboxylase degradation and total protein breakdown by lysosomotropic agents in 3T3-L1 cells. *Biochem. J.* 210:845–53
26. Chauhan, J., Dakshinamurti, K. 1986. Purification and characterization of human serum biotinidase. *J. Biol. Chem.* 261:4268–75
27. Chauhan, J., Dakshinamurti, K. 1987. Biotinidase as biotin-binding protein in human plasma. *Can. Fed. Biol. Soc.* 30:PA64 (Abstr.)
28. Craft, D. V., Goss, N. H., Chandramouli, N., Wood, H. G. 1985. Purification of biotinidase from human plasma and its activity on biotinyl peptides. *Biochemistry* 24:2471–76
29. Dakshinamurti, K., Bhagavan, H. N., eds. 1985. Biotin. *Ann. NY Acad. Sci.* 447:1–435
30. Dakshinamurti, K., Chalifour, L., Bhullar, R. P. 1985. Requirement for biotin and the function of biotin in cells in culture. *Ann. NY Acad. Sci.* 447:38–55
31. Dakshinamurti, K., Chauhan, J., Ebrahim, H. 1987. Intestinal absorption of biotin and biocytin in the rat. *Biosci. Rep.* 7:667–73
32. Desjardins, P. R., Dakshinamurti, K. 1971. Acetyl-CoA holocarboxylase synthesis in biotin-deficient rat adipose tissue. *Arch. Biochem. Biophys.* 142:292–98
33. 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

34. Du Vigneaud, W., Melville, D. B., Folkers, K., Wolf, D. E., Mozingo, R., et al. 1942. The stucture of biotin: A study of desthiobiotin. *J. Biol. Chem.* 146:475–85
35. Ebrahim, H., Dakshinamurti, K. 1986. A fluorometric assay for biotinidase. *Anal. Biochem.* 154:282–86
36. Eisenberg, M. A. 1973. Biotin: biogenesis, transport, and their regulation. *Adv. Enzymol.* 38:317–72
37. Eisenberg, M. A. 1985. Regulation of Biotin Operon in E. Coli. *Ann. NY Acad. Sci.* 447:335–49
38. Eisenberg, M. A., Prakash, O., Hsiung, S. C. 1982. Purification and properties of the biotin repressor. A bifunctional protein. *J. Biol. Chem.* 257:15167–73
39. Fisher, P. W. E., Goodridge, A. G. 1978. Coordinate regulation of acetyl coenzyme A carboxylase and fatty acid synthetase in liver cells of the developing chick *in vivo* and in culture. *Arch. Biochem. Biophys.* 190:332–44
40. Flint, D. J., Clegg, R. A., Vernon, R. G. 1983. Adipose tissue metabolism during early pregnancy in the rat: temporal relationship of changes in the metabolic activity, number of insulin receptors, and serum hormone concentration. *Arch. Biochem. Biophys.* 224:677–81
41. Freytag, S. O., Collier, K. J. 1984. Molecular cloning of a cDNA for human pyruvate carboxylase. Structural relationship to other biotin-containing carboxylases and regulation of mRNA content in differentiating preadipocytes. *J. Biol. Chem.* 259:12831–37
42. Freytag, S. O., Utter, M. F. 1980. Induction of pyruvate carboxylase apoenzyme and holoenzyme in 3T3-L1 cells during differentiation. *Proc. Natl. Acad. Sci. USA* 77:1321–25
43. Freytag, S. O., Utter, M. F. 1983. Regulation of the synthesis and degradation of pyruvate carboxylase in 3T3-L1 cells. *J. Biol. Chem.* 258:6307–12
44. Giffhon, S., Katz, N. R. 1984. Glucose-dependent induction of acetyl-CoA carboxylase in rat hepatocyte cultures. *Biochem. J.* 221:343–50
45. Gillevet, P. M., Dakshinamurti, K. 1982. Rat liver fatty-acid synthesizing complex. *Biosci. Rep.* 2:841–48
46. Gillevet, P. M., Dakshinamurti, K. 1983. Phosphorylation of proteolytically-nicked rat hepatic acetyl-CoA carboxylase. *Int. J. Biochem.* 15: 1361–65
47. Gompertz, D., Bartlett, K., Blair, D., Stern, C. M. M. 1973. Child with a defect in leucine metabolism associated with β-hydroxyisovelaric aciduria and β-methylcrotonylglycineuria. *Arch. Dis. Child* 48:975–77
48. Goodall, G. J., Johannsen, W., Wallace, J. C., Keech, D. B. 1985. Sheep liver propionyl-CoA carboxylase: purification and some molecular properties. *Ann. NY Acad. Sci.* 447:396–97
49. Goodridge, A. G., Adelman, T. G. 1976. Regulation of malic enzyme synthesis by insulin, triiodothyronine, and glucagon in liver cell cultures. *J. Biol. Chem.* 251:3027–32
50. Gore, J., Hoinard, C. 1987. Evidence of facilitated transport of biotin by hamster enterocytes. *J. Nutr.* 117:527–32
51. Goss, N. H., Wood, H. G. 1984. Formation of Nε-(biotinyl) lysine in biotin enzymes. *Methods Enzymol.* 107:261–78
52. Gravel, R. A., Lam, K. F., Mahuran, D., Kronis, A. 1980. Purification of human liver-propionyl-CoA carboxylase by carbon tetrachloride extraction and monomeric avidin chromatography. *Arch. Biochem. Biophys.* 201:669–73
53. Gravel, R. A., Lam, K.-F., Scully, K. J., Hsia, Y. E. 1977. Genetic complementation of proionyl-CoA carboxylase deficiency in cultured human fibroblasts. *Am. J. Hum. Genet.* 29:378–88
54. Gravel, R. A., Robinson, B. H. 1985. Biotin-dependent carboxylase deficiences (propionyl-CoA and pyruvate carboxylase). *Ann. NY Acad. Sci.* 447:225–34
55. Green, H., Kehinde, O. 1974. Subline of mouse 3T3 cells that accumulate lipid. *Cell* 1:113–16
56. Green, H., Kehinde, O. 1975. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. *Cell* 5:19–27
57. Guerre-Millo, M., Leturque, A., Girard, J., Lavau, M. 1985. Increased insulin sensitivity and responsiveness of glucose metabolism in adipocytes from female versus male rats. *J. Clin. Invest.* 76:109–16
58. Gyorgy, 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
59. Hardie, D. G., Carling, D., Ferrari, S., Guy, P. S., Aitkin, A. 1986. Characterization of the phosphorylation of

rat mammary ATP-citrate lyase and acetyl-CoA carboxylase by $Ca^{2+}$ and calmodulin-dependent multiprotein kinase and $Ca^{2+}$ and calmodulin-dependent multiprotein kinase and $Ca^{2+}$ and phospholipid-dependent protein kinase. *Eur. J. Biochem.* 157:555–61

60. Hardie, D. G., Cohen, P. 1978. Purification and physiochemical properties of fatty acid synthetase and acetyl-CoA carboxylase from lactating rabbit mammary gland. *Eur. J. Biochem.* 92:25–34
61. Harris, S. A., Wolf, D. E., Mozingo, R., Folkes, K. 1943. Synthetic biotin. *Science* 97:447–48
62. Haystead, A. J., Hardie, D. G. 1986. Both insulin and epidermal growth factor stimulate lipogenesis and acetyl-CoA carboxylase activity in isolated adipocytes. *Biochem. J.* 234:279–84
63. Heard, G. S., Grier, R. E., Weiner, D. L., Secor McVoy, J. R., Wolf, B. 1985. Biotinidase—a possible mechansim for the recycling of biotin. *Ann. NY Acad. Sci.* 447:400 (Abstr.)
64. Himes, R. H., Young, D. L., Ringelmann, E., Lynen, F. 1963. The biochemical function of biotin. V. Further studies on β-methylcrotonyl CoA carboxylase. *Biochem. Z.* 337:48–61
65. Holland, R., Hardie, D. G. 1985. Both insulin and epidermal growth factor stimulates fatty acid synthesis and increase phosphorylation of acetyl-CoA carboxylase and ATP-citrate lyase in isolated hepatocytes. *FEBS Lett.* 181: 308–12
66. Hopner, T., Knappe, J. 1965. Einbau von biotin in β-methylcrotonyl-CoA carboxylase durch holocarboxylase-synthetase. *Biochem. Z.* 342:190–206
67. Howard, P. K., Shaw, J., Otsuka, A. J. 1985. Nucleotide sequence of the bir A gene encoding the biotin operon repressor and biotin holoenzyme synthetase function of *Escherichia coli*. *Gene* 35:321–31
68. Ingebritsen, T. S., Blair, J., Guy, P., Witters, L., Hardie, D. G. 1983. The protein phosphates involved in cellular regulation. 3. Fatty acid synthesis, cholesterol synthesis and glycolysis/gluconeogenesis. *Eur. J. Biochem.* 132:275–81
69. Ingebritsen, T. S., Cohen, P. 1983. The protein phosphorylation involved in cellular regulation. 1. Classification and substrate specificity. *Eur. J. Biochem.* 132:255–61
70. Inoue, H., Lowenstein, J. M. 1972. Acetyl coenzyme A carboxylase from rat liver. Purification and demonstration of different subunits. *J. Biol. Chem.* 247: 4825–32
71. Kalousek, F., Darigo, M. D., Rosenberg, L. E. 1980. Isolation and characterization of propionyl-CoA carboxylase from normal human liver. *J. Biol. Chem.* 255:60–65
72. Katsurada, A., Fukuda, H., Iritani, N. 1986. Effect of dietary nutrients on substrate and effector levels of lipogenic enzymes, and lipogenesis from tritiated water in rat liver. *Biochim. Biophys. Acta* 878:200–8
73. Katsurada, A., Iritani, N., Fukuda, H., Noguchi, T., Tanaka, T. 1986. Effect of dietary nutrients on lipogenic enzymes mRNA activities in rat liver during induction. *Biochim. Biophys. Acta* 877: 350–58
74. Katz, N. R., Ick, M. 1981. Induction of acetyl-CoA carboxylase in primary rat hepatocyte cultures by glucose and insulin. *Biochem. Biophys. Res. Commun.* 100:703–9
75. Keeton, B. R., Moosa, A. 1976. Organic aciduria. Treatable cause of floppy syndrome. *Arch. Dis. Child* 51:636–38
76. Kim, K-H. 1983. Regulation of acetyl-CoA carboxylase. *Curr. Top. Cell. Regul.* 22:143–76
77. Kitajima, K., Tashiro, S.-I., Numa, S. 1975. Acetyl-coenzyme A carboxylase in cultured hepatocytes. Effect of exogenous fatty acid on the content, synthesis, and degradation of the enzyme. *Eur. J. Biochem.* 54:373–83
78. Klandorf, H., Clark, B. L., Brown, J. 1987. Altered glucagon release in biotin deficiency. *Gen. Comp. Endocrinol.* 65:133–40
79. Knappe, J., Brummer, W., Biederbick, K. 1963. Reinigung and eigenschaften der biotinidase aus schweinenieren und *lactobacillus casei*. *Biochem. Z.* 338: 599–613
80. Kogl, F., Tonnis, B. 1936. Uber das Bios-Problem. Darstellung von krystallisiertem Biotin aus Eigelb. *Z. Physiol. Chem.* 242:43–73
81. Kosow, D. P., Lane, M. D. 1962. Propionyl holocarboxylase formation: covalent bonding of biotin to apocarboxylase lysyl-amino groups. *Biochem. Biophys. Res. Commun.* 7:439–43
82. Krakower, G. R., Kim, K.-H. 1982. Purification and properties of acetyl-CoA carboxylase phosphatases. *J. Biol. Chem.* 256:2408–13
83. Krause, K.-H., Bonjour, J.-P., Berlit, P., Kochen, W. 1985. Biotin status of epileptics. *Ann. NY Acad. Sci.* 447:297–313
84. Kraus, J. P., Firgaira, F., Novotny, J.,

Kalousek, F., Williams, K. R., et al. 1986. Coding sequence of the precursor of the β-subunit of rat propionyl-CoA carboxylase. *Proc. Natl. Acad. Sci. USA* 83:8049–53

85. Kraus, J. P., Kalousek, F., Rosenberg, L. E. 1983. Biosynthesis and mitochondrial processing of the β-subunit of propionyl coenzyme A carboxylase from rat liver. *J. Biol. Chem.* 258:7245–48
86. Kraus, J. P., Williamson, C. L., Firgaira, F. A., Yang-Feng, T. L., Munke, M., et al. 1986. Cloning and screening with nanogram amounts of immunopurified mRNAs: cDNA cloning and chromosomal mapping of cystathionine β synthetase and the β subunit of propionyl-CoA carboxylase. *Proc. Natl. Acad. Sci. USA* 83:2047–51
87. Kuri-Harcuch, W., Green, H. 1976. Increasing activity of enzymes on pathway of triacylglycerol synthesis during adipose conversion of 3T3 cells. *J. Biol. Chem.* 252:2158–60
88. Kuri-Harcuch, W., Wise, L. S., Green, H. 1978. Interruption of the adipose conversion of 3T3 cells by biotin deficiency: differentiation without triglyceride accumulation. *Cell* 14:53–59
89. Lamhonwah, T. A. M., Barankiewicz, T., Willard, H. F., Mahuran, D., Quan, F., Gravel, R. A. 1985. Propioicacidemia: Absence of alpha chain mRNA in pccA complementation group. *Am. J. Hum. Genet.* 37:A164 (Abstr.)
90. Lamhonwah, A., Barankiewicz, T. J., Willard, H. F., Mahuran, D. J., Quan, F., Gravel, R. A. 1986. Isolation of cDNA clones coding for the α and β chains of human propionyl-coA carboxylase: chromosomal assignments and DNA polymorphisms associated with PCCA and PCCB genes. *Proc. Natl. Acad. Sci. USA* 83:4864–68
91. Lane, M. D., Moss, J., Polakis, S. E. 1974. Acetyl coenzyme A carboxylase. *Curr. Top. Cell. Regul.* 8:139–95
92. Lane, M. D., Young, D. L., Lynen, F. 1964. The enzymatic synthesis of holotranscarboxylase from apotranscarboxylase and (+) biotin. I. Purification of the apoenzyme and synthetase; characteristics of the reaction. *J. Biol. Chem.* 239:2858–64
93. Lee, K.-L., Darke, P. L., Kenney, F. T. 1977. Role of coenzyme in aminotransferase turnover. *J. Biol. Chem.* 252: 4958–61
94. Lehnert, W., Niederhoff, H., Junker, A., Saule, H., Frasch, W. 1979. A case of biotin-responsive 3-methylcrotonylglycine- and 3-methylhydroxyisovaleric aciduria. *Eur. J. Pediatr.* 132:107–14
95. Lent, B., Kim, K.-H. 1983. Phosphorylation and activation of acetyl-coenzyme a carboxylase kinase by the catalytic subunit of cyclic AMP-dependent protein kinase. *Arch. Biochem. Biophys.* 225:972–78
96. 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
97. Lyen, F., Rominger, K. L. 1963. Formation of acetyl CoA carboxylase from its apocarboxylase. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 22:537 (Abstr.)
98. Mackall, J. C., Lane, M. D. 1977. Role of pyruvate carboxylase in fatty acid synthesis: alterations during preadipocyte differentiation. *Biochem. Biophys. Res. Commun.* 79:720–25
99. Mackall, J. C., Student, A. K., Polakis, S. E., Lane, M. D. 1976. Induction of lipogenesis during differentiation in a "preadipocyte" cell line. *J. Biol. Chem.* 251:6462–64
100. Majerus, P. W., Kilburn, E. 1969. Acetyl coenzyme A carboxylase. The role of synthesis and degradation in regulation of enzyme levels in rat liver. *J. Biol. Chem.* 244:6254–62
101. Maloy, W. L., Bowien, B. U., Zwolinski, G. K., Kumar, G. K., Wood, H. G., et al. 1979. Amino acid sequence of the biotinyl subunit from transcarboxylase. *J. Biol. Chem.* 254:11615–22
102. McClure, W. R., Lardy, H. A. 1971. Rat liver pyruvate carboxylase. IV. Factors affecting the regulation *in vivo*. *J. Biol. Chem.* 246:3591–96
103. McClure, W. R., Lardy, H. A., Kneifel, H. P. 1971. Rat liver pyruvate carboxylase. I. Preparation, properties, and cation specificity. *J. Biol. Chem.* 246: 3569–78
104. Mistry, S. P., Dakshinamurti, K. 1964. Biochemistry of biotin. *Vitam. Horm.* 22:1–55
105. Mock, D. M., Baswell, D. L., Baker, H., Holman, R. T., Sweetman, L. 1985. Biotin deficiency complicating parenteral alimentation: diagnosis, metabolic repercussion, and treatment. *Ann. NY Acad. Sci.* 447:314–34
106. Moss, J., Lane, M. D. 1971. The biotin-dependent enzymes. *Adv. Enzymol.* 35:321–42
107. Moss, J., Lane, M. D. 1972. Acetyl coenzyme A carboxylase. III. Further studies on the relation of catalytic activity to polymeric state. *J. Biol. Chem.* 247:4944–51

108. Moss, J., Yamagishi, M., Kleinschmidt, A. K., Lane, M. D. 1972. Acetyl coenzyme A carboxylase. Purification and properties of the bovine adipose tissue enzyme. *Biochemistry* 11:3779–86
109. Munday, M. R., Haystead, T. A. J., Holland, R., Carling, D. A., Hardie, D. G. 1986. The role of phosphorylation/dephosphorylation of acetyl-CoA carboxylase in the regulation of mammalian fatty acid biosynthesis. *Biochem. Soc. Trans.* 14:559–62
110. Nakanishi, S., Numa, S. 1970. Purification of rat liver acetyl coenzyme A carboxylase and immunochemical studies on its synthesis and degradation. *Eur. J. Biochem.* 16:161–73
111. Nakanishi, S., Numa, S. 1971. Synthesis and degradation of liver acetyl coenzyme A carboxylase in genetically obese mice. *Proc. Natl. Acad. Sci. USA* 68: 2288–92
111a. National Research Council. 1980. *Recommended Dietary Allowances,* pp. 120–22. Washington, DC: Natl. Acad. Sci. 9th revised ed.
112. Nishimura, H., Yoshioka, K., Iwashima, A. 1984. A method for determining binding kinetics applied to thiamine-binding protein. *Anal. Biochem.* 139: 373–76
113. Nomoto, M., Narahashi, Y., Murakami, M. 1960. A proteolytic enzyme of *Streptomyces griseus.* VI. Hydrolysis of protein by *Streptomyces griseus* protease. *J. Biochem. (Tokyo)* 48:593
114. Nomoto, M., Narahashi, Y., Murakami, M. 1960. A proteolytic enzyme of *Streptomyces griseus* protease. *J. Biochem. (Tokyo)* 48:906
115. Ogiwara, H., Tanahe, T., Nikawa, J., Numa, S. 1978. Inhibition of rat liver acetyl coenzyme A carboxylase by palmitoyl coenzyme A. *Eur. J. Biochem.* 89:33–41
116. Otsuka, A., Abelson, J. 1978. The regulatory region of the biotin operon in *Escherichia coli. Nature* 276:689–94
117. Packman, S., Caswell, N. M., Baker, H. 1982. Biochemical evidence for diverse etiologies in biotin-responsive multiple carboxylase deficiency. *Biochem. Genet.* 20:17–28
118. Pispa, J. 1965. Animal biotinidase. *Ann. Med. Exp. Biol. Fenn.* 43(Suppl. 5):5–39
119. Ramakrishna, S., Benjamin, W. B. 1983. Rapid purification of enzymes of fatty acid biosynthesis from rat adipost tissue. *Prep. Biochem.* 13:475–88
120. Deleted in proof
121. 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
122. Rosenberg, L. E. 1983. In *The Metabolic Basis of Inherited Disease,* ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Frederickson, J. I. Goldstein, M. S. Brown, pp. 474–97. New York: McGraw-Hill
123. Rubin, C. S., Hirsch, A., Fung, C., Rosen, O. M. 1978. Development of hormone receptors and hormonal responsiveness *in vitro.* Insulin receptors and insulin sensitivity in the preadipocyte and adipocyte forms of 3T3-L1 cells. *J. Biol. Chem.* 253:7570–78
124. Rylatt, D. B., Keech, D. B., Wallace, J. C. 1977. Pyruvate carboxylase: isolation of the biotin-containing tryptic peptide and the determination of its primary sequence. *Arch. Biochem. Biophys.* 183: 113–22
125. Said, S. M., Redha, R. 1987. A carrier-mediated system for transport of biotin in rat intestine *in vitro. Am. J. Physiol.* 252:G52–G55
126. Salati, L. M., Clarke, S. D. 1986. Fatty acid inhibition of hormonal induction of acetyl-coenzyme A carboxylase in hepatocyte monolayers. *Arch. Biochem. Biophys.* 246:82–89
127. Scheiner, J., DeRitter, E. 1975. Biotin contents of feedstuffs. *J. Agric. Food Chem.* 23:1157–62
128. Schiele, U., Niedermeier, R., Sturzer, M., Lynen, F. 1975. Investigation of the structure of 3-methylcrotonyl-CoA carboxylase from *Archromobacter. Eur. J. Biochem.* 60:259–66
129. Schudt, C. 1979. Hormonal regulation of glucokinase in primary cultures of rat hepatocytes. *Eur. J. Biochem.* 98:77–82
130. Scrutton, M. D., Fung, C.-H. 1972. Pyruvate carboxylase from chicken liver: Effect of sulphate and other anions on catalytic activity and structural parameters. *Arch. Biochem. Biophys.* 150:636–47
131. Scrutton, M. C., Young, M. R. 1972. Pyruvate carboxylase. *Enzymes* 6:1–35
132. Shafrir, E., Bierman, E. L. 1981. Acetyl-CoA carboxylase activity in cultured human fibroblasts. Induction by insulin in relation to cell growth and triacylglycerol metabolism. *Biochim. Biophys. Acta* 663:432–45
133. Sorrell, M. F., Frank, O., Thomas, A. D., Aquino, A., Baker, H. 1971. Absorption of vitamins from large intestine *in vivo. Nutr. Rep. Int.* 3:143–48
134. Spence, J. T., Merrill, M. J., Pitot, H.

C. 1981. Role of insulin, glucose, and cyclic GMP in primary cultures of rat hepatocytes. Relationship between the regulation of glucokinase in cultured hepatocytes. *J. Biol. Chem.* 256:1598–1603

135. Spence, J. T., Pitot, H. C. 1982. Induction of lipogenic enzymes in lipogenesis and carbohydrate metabolism. *Eur. J. Biochem.* 128:15–20
136. Spencer, R. P., Brody, K. R. 1964. Biotin transport by small intestine of rat, hamster, and other species. *Am. J. Physiol.* 206:653–57
137. Student, A. K., Hsu, R. Y., Lane, M. D. 1980. Induction of fatty acid synthetase synthesis in differentiating 3T3-L1 preadipocytes. *J. Biol. Chem.* 255:4745–50
138. Sutton, M. R., Fall, R. R., Nervi, A. M., Alberts, A. M., Vagelos, P. R., Bradshaw, R. A. 1977. Amino acid sequence of *Escherichia coli* carboxyl carrier protein (9100). *J. Biol. Chem.* 252:3934–40
139. Sweetman, L. 1981. Two forms of biotin-responsive multiple carboxylase deficiency. *J. Inherit. Metab. Dis.* 4:53–54
140. Sweetman, L., Nyhan, W. L. 1986. Inheritable biotin-treatable disorders and associated phenomena. *Ann. Rev. Nutr.* 6:317–43
141. Sydenstricker, V. P., Singal, S. A., Briggs, A. P., DeVaughn, N. M., Isbell, H. 1942. Observations on the "egg white injury" in man and its cure with a biotin concentrate. *J. Am. Med. Assoc.* 118:1199–1200
142. Tanaka, K. 1981. New light on biotin deficiency. *N. Engl. J. Med.* 304:839–40
143. Thomson, R. C., Eakin, R. E., Williams, R. J. 1941. The extraction of biotin from tissues. *Science* 94:589–90
144. Utter, M. F., Barden, R. E., Taylor, B. L. 1975. Pyruvate carboxylase: an evaluation of the relationship between structures and mechanisms and between structure and catalytic activity. *Adv. Enzymol.* 42:1–72
145. Utter, M. F., Keech, D. B. 1960. Formation of oxaloacetate from pyruvate and $CO_2$. *J. Biol. Chem.* 235:PC17–PC18
146. Utter, M. F., Keech, D. B. 1963. Pyruvate carboxylase. I. Nature of the reaction. *J. Biol. Chem.* 238:2603–8
147. Walter, P. 1976. In *Gluconeogenesis: Its Regulation in Mammalian Species,* ed. R. W. Hanson, M. A. Mehlman, pp. 239–65. London: Wiley
148. 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
149. Weinberg, M. B., Utter, M. F. 1979. Effect of thyroid hormones on the turnover of rat liver pyruvate carboxylase and pyruvate dehydrogenase. *J. Biol. Chem.* 254:9492–99
150. Weinberg, M. B., Utter, M. F. 1980. Effect of streptozotocin-induced diabetes mellitus on the turnover of rat liver pyruvate carboxylase and pyruvate dehydrogenase. *Biochem. J.* 188:601–8
151. Weiner, D. L., Grier, R. E., Watkins, P., Heard, G. S., Wolf, B. 1983. Tissue origin of serum biotinidase; implication in biotinidase deficiency. *Am. J. Hum. Genet.* 34:56A
152. Weiss, G. H., Rosen, O. M., Rubin, C. S. 1980. Regulation of fatty acid synthetase concentration and activity during adipocyte differentiation. Studies on 3T3-L1 cells. *J. Biol. Chem.* 255:4751–57
153. Witters, L. A., Mendel, D. B., Colliton, J. W. 1987. Modulation of acetyl-CoA carboxylase by inhibition of IMP dehydrogenase: implication for insulin regulation. *Arch. Biochem. Biophys.* 252:130–35
154. Witters, L. A., Moriarity, D., Martin, D. B. 1979. Regulation of hepatic acetyl coenzyme A carboxylase by insulin and glucagon. *J. Biol. Chem.* 254:6644–49
155. Wolf, B. 1978. Biochemical characterization of mutant propionyl-CoA carboxylases from two minor genetic complementation groups. *Biochem. Genet.* 17:703–7
156. Wolf, B., Feldman, G. L. 1982. The biotin-dependent carboxylase deficiencies. *Am. J. Hum. Genet.* 34:699–716
157. 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
158. Wolf, B., Grier, R. E., McVoy, J. R. S., Heard, G. S. 1985. Biotinidase deficiency: a novel vitamin recycling defect. *J. Inherited Metab. Dis.* 8(Suppl. 1):53–58
159. 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
160. Wolf, B., Heard, G. S., McVoy, J. R. S., Raetz, H. M. 1984. Biotinidase de-

ficiency: the possible role of biotinidase in the processing of dietary protein-bound biotin. *J. Inherited Metab. Dis.* 7(Suppl. 2):121–22

161. Wolf, B., Hsia, Y. E., Rosenberg, L. E. 1978. Biochemical differences between mutant propionyl-CoA carboxylases from two complementation groups. *Am. J. Hum. Genet.* 30:455–64
162. 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
163. Wolf, B., McVoy, J. S. 1983. A sensitive radioassay for biotinidase activity: Deficient activity in tissues of serum biotinidase-deficient individuals. *Clin. Chim. Acta* 135:275–81
164. Wolf, B., Willard, H. F., Rosenberg, L. E. 1980. Kinetic analysis of genetic complementation in heterokaryons of propionyl CoA carboxylase-deficient human fibroblasts. *Am. J. Hum. Genet.* 32:16
165. Wood, H. G., Barden, R. E. 1977. Biotin enzymes. *Ann. Rev. Biochem.* 46:385–413
166. Wood, H. G., Harmon, F. R., Wuhr, B., Hubner, K., Lynen„ F. 1980. Comparison of the biotinidase of apotranscarboxylase and its aposubunits. Is assembly essential for biotinidase. *J. Biol. Chem.* 7:397–409
167. Wood, H. G., Kumar, G. K. 1985. Transcarboxylase: its quaternary structure and the role of the biotinyl subunit in the assembly of the enzyme and in catalysis. *Ann. NY Acad. Sci.* 447:1–21
168. Yatsidis, H., Koutsinas, D., Agrayannis, B., Papastephanidis, C., Francos-Phemanis, M., Delatala, Z. 1984. Biotin in the management of uremic neurologic disorders. *Nephron* 36:183–85
169. Yeh, L. A., Song, C. S., Kim, K.-H. 1981. Coenzyme A activation of acetyl-CoA carboxylase. *J. Biol. Chem.* 256: 2289–96
170. Zakim, D., Pardini, R. S., Herman, R. H., Sauberlich, J. 1967. Mechanism for the differential effects of higher carbohydrate diets on lipogenesis in rat liver. *Biochim. Biophys. Acta* 144:242–51