VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

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Preface

The Editors take pleasure in presenting this eighteenth volume of Vitamins and Hormones.

An unusual departure from previous policy is the inclusion of the papers delivered at the Symposium on Vitamin A and Metabolism, held in honor of Professor P. Karrer at Bürgenstock, Switzerland, on May 23, 24, and 25, 1960. This does not represent a permanent change in editorial policy, although it is not impossible that it might be repeated if another symposium so specifically in our field of interest and of equal breadth and importance were to take place. Rather it should be considered as an experiment, on the results of which future policy may depend. The editors would be pleased to receive readers' comments upon it. It had been hoped that this symposium could be published within a few months of its occurrence by including it herewith without delaying the appearance of the volume. We regret that this has proved impossible.

Volume 18 contains four chapters on vitamins and three on hormones, in addition to those in the Vitamin A Symposium. All of these chapters are critical reviews in which completeness of coverage has not been allowed to swamp that personal approach which makes a chapter readable. We feel that they are important contributions to understanding in this field.

In his remarks (p. 571) at the close of the Vitamin A Symposium, Professor Karrer quotes Goethe as saying: "Science is a great musical fugue, in which the voice of every nation finds its expression," and goes on to ask "Is there anything better than these free discussions from country to country, from continent to continent, which do not serve to subject humanity nor impose power, but which are aimed at unveiling the secrets of nature and extending our understanding of the universe?" These thoughts are, it seems to us, particularly appropriate for Vitamins and Hormones, since this publication is international in authorship, supranational in subject matter and world-wide in distribution.

The Editors wish to express warm appreciation to the 29 authors from five countries who have contributed to Volume 18. It is our eager hope that they will feel a deep satisfaction in having performed this important service for their colleagues.

ROBERT S. HARRIS
DWIGHT J. INGLE

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Physiology and Biochemistry of Biotin

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I. Introduction

The aim of this chapter is to discuss the principal characteristics of the physiology and biochemistry of biotin. We have considered not only new data which have appeared since the review by Lichstein (1951), but also recalled earlier facts which did not come within the compass of that author's review.

The present work is not complete. In particular, we have not considered the problems of the specificity of action of biotin, the role of biotin in resistance to infectious diseases, and the improbable connection between

biotin and cancer. These questions have been dealt with in detail by György (1954).

In considering the metabolic activities of biotin, a parallel has been established, whenever possible, between the participation of the vitamin in microbial metabolism and in animal metabolism. In this way it is possible to show clearly the universal or limited ability of biotin to participate in certain metabolic operations, to demonstrate the common or separate processes by which biotin intervenes in these operations in microorganisms and in animals, and to point out the gaps relative to the possible implication of biotin in these two metabolisms.

Certain other aspects of the action of biotin come solely within the compass of animal physiology, e.g. the problems of the relationship between biotin and the endocrine glands, the nervous system and the skin. Although they are of limited bearing, it is essential to understand these relationships in order to be able to define the properties of biotin.

II. The Metabolic Activities of Biotin

1. Carbohydrate Metabolism

Biotin takes part in the following reactions: (1) the Wood-Werkman reaction proceeding to the reversible conversion of pyruvic acid to oxalacetic acid and vice versa; (2) conversion of malic acid to pyruvic acid in the presence of malic enzyme; (3) interconversion of succinic acid and propionic acid; (4) conversion of oxalosuccinic acid to α-ketoglutaric acid; (5) different enzymatic reactions of dehydrogenation, such as that of succinic acid. For the sake of clarity these reactions have been grouped with carbohydrate metabolism, but it is obvious that several of them have a much wider implication and participate in the intermediate metabolism of ternary chains in general.

The first four reactions are carboxylation and decarboxylation reactions. This fact is of prime importance. Indeed, while certain biochemical actions of biotin are still obscure, it seems incontestable that this vitamin participates in many carboxylation and decarboxylation reactions not only in carbohydrate metabolism, but also, as will be seen, in lipid, protein and nucleic acid metabolism.

For some of the above five reactions, the participation of biotin has been demonstrated until now only in microorganisms; for others, the vitamin plays a general role, since it has also been recognized in the tissues of mammals and birds. Among the latter reactions, that of Wood-Werkman is of fundamental importance. By its participation in this reaction, biotin—along with thiamine, riboflavin, niacinamide, and pantothenic acid—plays an indispensable role in the oxidation cycle of the ter-

nary chains. This role is specifically to provide the oxalacetic acid for this cycle.

The participation of biotin in the reactions of carbohydrate metabolism enumerated above has been thoroughly discussed by Lichstein (1951). We mention here only the new facts, indeed few in number, which have appeared since 1951.

a. Probable Coenzyme Nature of Biotin in Oxalacetate Decarboxylase. It seems probable that biotin participates as a coferment in the five reactions mentioned above. But this presumption will be established only with the isolation of biotin from absolutely pure enzymes. The only research carried out in this field has been concerned with the Wood-Werkman reaction. Lichstein (1955, 1957) succeeded in establishing a very significant correlation between the degree of purity of oxalacetate decarboxylase extracted from the liver of the chick, and the quantity of biotin which is freed by hydrolysis. The purest enzyme preparation contains 3 mμg. biotin per milligram protein, which represents approximately 1 mole biotin per 10^8 g. of protein (Lichstein, 1957). However Lichstein's own opinion is that these results are only indicative and that the purification and analysis of the constitution of oxalacetate decarboxylase must be carried out before it can be stated definitely that biotin is its coenzyme.

b. Interconversion of Succinic Acid to Propionic Acid. i. Conversion of propionic acid to succinic acid. (1) Animal metabolism: The principal stages of the enzymatic oxidation of propionic acid in animal tissues have been established largely by the work of Ochoa (Flavin and Ochoa, 1957; Flavin *et al.*, 1957; Beck *et al.*, 1957) and Lardy (Lardy and Peanasky, 1953; Lardy and Adler, 1956) as follows:

(i) $\text{propionate} \xrightarrow[\text{enzyme 1}]{\text{ATP + CoA}} \text{propionyl CoA}$

(ii) $\text{propionyl CoA} \xrightarrow[\text{enzyme 2}]{\text{ATP + CO}_2} \text{isosuccinyl CoA + ADP + PO}_4\text{H}_3$

(iii) $\text{isosuccinyl CoA} \xrightarrow[\text{isomerase}]{} \text{succinyl CoA}$

(iv) $\text{succinyl CoA} \longrightarrow \text{succinate}$

(v) $\text{succinate} \xrightarrow[\text{via citric acid cycle}]{} \text{oxidation}$

Biotin deficiency decreases the capacity of rat liver mitochondria extract to synthesize succinate from propionate and from $NaHC^{14}O_3$. This capacity is in no way restored by the *in vitro* addition of biotin or biocytin; on the other hand, it reappears very rapidly after administration of biotin to the living deficient animal (Lardy and Adler, 1956).

The precise stage of the intervention of biotin in the conversion of propionic acid to succinic acid still remains to be determined, however. This determination is all the more delicate because the mechanism of re-

action (ii) in particular is still obscure. This reaction is probably catalyzed by a single enzyme which is referred to as a propionyl carboxylase. This purified enzyme contains no biotin (Tietz and Ochoa, 1959). Thus, the reported function of biotin in propionate carboxylation remains unexplained.

(2) Metabolism of microorganisms: *Propionibacterium pentosaceum* (Barban and Ajl, 1951; Delwiche *et al.*, 1953) and *Micrococcus lactilyticus* (Whiteley, 1953b) also convert propionic acid to succinic acid, but it has not been possible to determine whether the mechanism is the same as in animal tissues.

ii. Conversion of succinic acid to propionic acid. Metabolism of microorganisms: Since the initial work of Delwiche (1950) and of Lichstein (1950) related by the latter in 1951, supplementary information has been published on the decarboxylation of succinic acid to propionic acid and the part that biotin plays in it. This reaction, according to Whiteley (1953a), is the principal pathway for the formation of propionic acid in *P. pentosaceum*, *M. lactilyticus*, and other microccoci. The conversion of succinic acid to propionic acid consists of the following two enzymatic operations (Delwiche *et al.*, 1953; Whiteley, 1953a,b; Chambers and Delwiche, 1954):

(i) $$\text{succinate} \xrightarrow{\text{ATP} + \text{CoA}} \text{succinyl CoA}$$

(ii) $$\text{succinyl CoA} \xrightarrow[\text{enzyme}]{\text{biotin ?}} \text{propionyl CoA} + CO_2$$

But no mention is made of the stage of the isosuccinyl-CoA step or of the presence of adenosine triphosphate (ATP) in phase (ii) of the reaction (Whiteley, 1953a,b). It is therefore not yet possible to decide whether these two observations are sufficient to establish the real differences between bacterial metabolism and animal metabolism in the interconversion of succinic acid to propionic acid. If the reaction converting succinic acid to propionic acid in microorganisms seems less complex than the one converting propionic acid to succinic acid in higher animals, it may be simply because it has not been as closely studied.

Seeking to determine exactly the site of biotin involvement in the conversion by microorganisms of succinic acid to propionic acid, Chambers and Delwiche (1954) showed that the vitamin does not participate in the first stage of the reaction. It would then seem that biotin is not involved either directly or indirectly in the formation of succinyl coenzyme A. It must, therefore, be involved in the second phase of the reaction, but its exact role has not yet been defined. Does it participate directly in the formation of the apoenzyme or the coenzyme of the succinyl CoA decarboxylase, or indirectly in the synthesis of the protein fraction of this

enzyme? These hypotheses were proposed by Chambers and Delwiche (1954).

Thus, the precise role of biotin in succinic acid-propionic acid interconversion is not known, either in animals or in microorganisms.

Lichstein (1958) stresses that biotin deficiency decreases the ability of *P. pentosaceum* both to decarboxylate succinic acid to propionic acid and to ferment glucose. Under these conditions, the glucose fermentation is restored by adding either biotin or desthiobiotin or oxybiotin to the medium; but the latter derivative stimulates the decarboxylation of succinic acid only weakly compared with biotin or desthiobiotin, which are equally effective. It might be that oxybiotin in particular is converted to an oxybiotin coenzyme which has relatively poor ability to serve as a cofactor for the succinate decarboxylase system but is fully capable of replacing the biotin coenzyme in the fermentation of glucose. These different considerations led Lichstein (1958) to the very interesting suggestion that there might be several coenzyme forms of biotin, in the same way that there are several coenzyme forms of niacinamide and pyridoxine.

c. Hexokinase. A culture of *Leuconostoc mesenteroides*, which does not require biotin when its medium contains saccharose, needs this vitamin if the medium contains a monosaccharide (Carlson and Whiteside-Carlson 1949). In the same way, a biotin-deprived culture of *Saccharomyces cerevisiae* (1939 strain) continues to grow moderately if the medium contains saccharose, but does not grow at all if it contains glucose (Williams *et al.*, 1957).

On the basis of these observations, Williams *et al.* (1957) proposed that biotin may control the initial stage of glucose catabolism, i.e. phosphorylation catalyzed by hexokinase (Fig. 1).

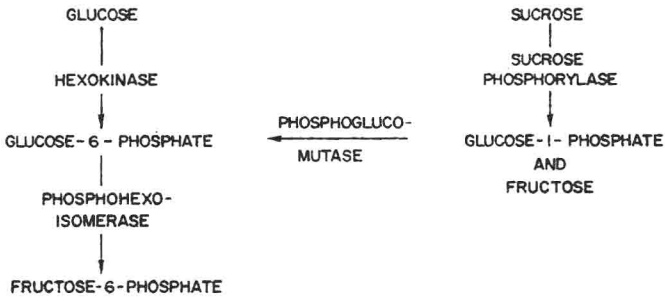

FIG. 1. Initial steps in the catabolism of glucose and sucrose. According to Williams *et al.* (1957).

The authors confirmed their hypothesis by showing that the phosphorylation velocity of the 2-deoxy-D-glucose is greatly reduced in a cell-free

extract of *S. cerevisiae* deficient in biotin. Strauss and Moat (1958) give the following supplementary information: glucose-6-phosphate or fructose-6-phosphate fermentation, is independent of biotin in the same way as hexose diphosphate; the activity of phosphoglucoisomerase and that of glucose-6-phosphate dehydrogenase are also independent of biotin.

These facts taken together indicate that there is a close relationship between biotin and hexokinase. The hexokinase reaction involves the dissociation of a proton from a hydroxyl group of glucose before phosphorylation. Possibly in the enzymatic systems in which biotin plays a part, it might, according to the hypothesis of Lichstein (1951), play a unique role in the hydrogen transport involved in these reactions. The mode of action of biotin might be, in the case of hexokinase, as Strauss and Moat suggested, to aid the dissociation of a hydrogen atom from an otherwise undissociable hydroxyl group of glucose. Strauss and Moat (1958) have gone still further in determining the link between biotin and hexokinase activity and have shown that the addition of free biotin *in vitro* restores the weakened hexokinase activity of biotin-deficient yeast extract cells under different conditions. This is, moreover, an exceptional observation in the biochemistry of biotin for most of the other enzymatic reactions that are weakened by biotin deficiency cannot be stimulated by the *in vitro* addition of this vitamin. It must be pointed out that it is this fact which casts doubt on the coenzyme nature of biotin in these enzymatic reactions.

The functional relationship between biotin and hexokinase shown above in *S. cerevisiae* strain 139 has never been observed in *P. pentosaceum*, strain E 214, although biotin deficiency reduces the ability of this microorganism to ferment glucose (Lichstein, 1958). In a mutant strain of *Escherichia coli* (23358) this functional relationship does not appear either (Ferguson and Lichstein, 1957). On the contrary, the presence of glucose triples or quintuples the growth of *E. coli* 23358 when this microorganism is deficient or partially deficient in biotin. The mechanism of this stimulating action is still unknown; it cannot be explained by a synthesis of biotin since the content of this vitamin in this microorganism stays the same in the presence or absence of glucose.

The role of biotin in the phosphorylation of glucose in yeast perhaps conforms with a more general observation of Sytinskaja (1956) that oxidative phosphorylation is reduced in the liver extracts of biotin-deficient rats. The administration of the vitamin only 90 minutes prior to killing restores phosphorylation to normal.

d. In Vivo Carbohydrate Metabolism of the Rat. i. Pyruvic acid level in blood. It is undeniable that biotin deficiency produces an important increase in blood pyruvic acid. T. Terroine (1956a) observed it to be dou-