# VITAMINS AND HORMONES

## ADVANCES IN RESEARCH AND APPLICATIONS



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## Editors' Preface

The success of volume I of Vitamins and Hormones, in spite of the difficulties imposed by war conditions, has shown the need that exists for critical and intelligent reviews of this field. The scattering of the literature through a wide variety of journals, the inevitable variation in the quality of the work, and the variety of interests represented, ranging through physics and chemistry to pathology and clinical medicine, make the task of the reviewer a difficult one and the value of the review correspondingly greater.

The Editors have felt that the authors should be free to exercise their judgment as to omission and selection of material, keeping in mind the aim of presenting a balanced picture of the development and current status of each subject. This is particularly true of fields of work which include numbers of inconclusive experiments. The two articles in the present volume relating to cancer provide an example of different approaches to this problem; in one the authors have aimed at discussing whatever work has been done, at the same time making clear the uncertain nature of some of it, while in the other only the best established results have been considered at all. Each author has difficulties of his own special kind to face, and the Editors wish to express their appreciation of the immense amount of work and thought which has gone into the preparation of all of the articles.

The policy of including complete subject and author indexes has been continued in the present volume, and it is believed that these indexes will add materially to the reference value of these yearly volumes. The Editors will welcome suggestions for improvement.

To some readers a volume like the present may appear to be essentially a series of complete but disconnected articles rather than an integrated whole. This must to a degree be true of any group of scientific reviews. In so far as it may be due to any real lack of correlation between ideas and results in different fields of endeavor, the bringing together of these reviews under one cover should be a stimulus toward closer interdependence of workers in distinct but related subjects. If such an integration is promoted, these volumes will have made a useful contribution to progress in the field of Vitamins and Hormones.

KENNETH V. THIMANN
ROBERT S. HARRIS

July, 1944

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BY E. W. MCHENRY AND MADELEINE L. CORNETT

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## I. INTRODUCTION

In the interpretation of the title of this review the authors have used Lusk's definition of anabolism (1): the construction of higher substances from lower ones. On this basis, the review deals with the possible effects of various vitamins upon the formation of complex lipids from much simpler molecules derived by metabolic processes from carbohydrates and proteins.

A number of recent reviews have dealt with the synthesis of lipids and with the effects of vitamins upon the synthesis and metabolism of lipids. Particularly valuable have been the excellent reviews by Longenecker (2), by Best and Lucas (3), by Bollman (4), and by Mitchell (5).

## II. CLASSICAL DEMONSTRATIONS OF FAT FORMATION

During the past century the process of lipogenesis has been demonstrated repeatedly. Although Boussingault and Persoz had described fat formation in geese (6), Lawes and Gilbert (7) are responsible for the first evidence clearly indicative of such a process in animals. Analyses of carbohydrate, fat, and protein of the food consumed by pigs, as well as of the resultant composition of the carcasses, served to demonstrate that extensive formation of lipid material from carbohydrate and protein had taken place. These observations confirmed the opinion earlier expressed by Liebig (8) that fat could be synthesized in the animal body.

In 1886, Rubner (9) described lipogenesis in dogs and subsequently Rosenfeld (10) and also Morgulis and Pratt (11) contributed observations on fat synthesis. The gaseous exchange experiments carried out by the latter, drew attention to the elevated respiratory quotient associated with active fat formation.

The synthesis of lipid material has also been shown to occur in bacterial and yeast cultures, in seeds, and in certain lower animals. In 1908, Weinland (12) described fat formation by macerated larvae incubated in a peptone medium. Beebe and Buxton (13) reached similar conclusions as a result of studies on *Bacillus pyocyaneus;* the pellicle formed by cultures of the organism after an incubation period of three weeks yielded a large amount of ether-extractable material.

More recent researches have indicated the extent to which this important metabolic process may function. In 1925, Wierzuchowski and Ling (14) corroborated the observation of Rapport and associates (15) on fat formation from carbohydrate by "well-nourished" swine. It was pointed out that lipid material was synthesized to the extent of 1% of the body weight daily. The contributions of Anderson and Mendel (16) on rats and of Ellis, *et al.* (17, 18) on hog fattening are in harmony with the earlier observations. Benedict and Lee (19), in a detailed study of fat formation in forced-fed geese, have emphasized the high degree to which lipogenesis may predominate in the metabolic processes of animals possessing a disposition to fat formation. Schoenheimer, *et al.* (20, 21) have demonstrated the rapidity with which lipogenesis occurs, and both Longenecker (22), and Hoagland and Snider (23) provided conclusive evidence of lipid formation from amino acids.

Although it had often been suggested that fat synthesis is essentially, if not exclusively, a hepatic function, only rather recently has satisfactory experimental support for this view become available. Bernhard and Schoenheimer (21) drew attention to the extreme rapidity of turnover of liver fatty acids, and Winter, *et al.* (24) described the failure of fat synthesis in Eck fistula dogs. Similar conclusions may be drawn from experiments of Barrett, Best, and Ridout (25), McHenry (26), and Stetten and Schoenheimer (27).

It is of interest that Hildesheim and Leathes (28) reported the formation of fat in minced dog, rabbit, and pig livers *in vitro*. However, their results were not considered conclusive since added carbohydrate did not stimulate lipid accumulation.

In investigations prior to 1910 and, indeed, in many subsequent to this date, no attention was paid to the vitamin content of the experimental diets. More recent investigations have indicated the essential relations of some vitamins to fat synthesis and have explained why certain foods proved to be lipogenic in the earlier work.

## III. The Synthesis of Fat from Carbohydrate

In 1936, Whipple and Church (29) extended observations made previously by Sure and associates (30, 31, 32), by Graham and Griffith (33), and by Mitchell (34) that vitamin $B_1$ had a specific effect upon body weight, as determined by using isocaloric feeding. In addition to repeating the earlier observations upon body weight, Whipple and Church analyzed the animal bodies and secured information regarding the reasons for the increase in weight. They found that, of the difference in weight between pair fed rats, 51% was due to deposition of fat and the balance to water. Since the animals had been maintained on a fat-free diet, and since the only difference in treatment of the two groups was the supply of thiamin to one group, Whipple and Church concluded that thiamin plays a rôle in the synthesis of fat in the animal body. In the following year, they reported (35) a marked difference in respiratory quotient between two isocalorically fed groups of rats, one of which received thiamin; the latter had a quotient well above unity, thus strengthening the hypothesis that thiamin brings about fat formation.

The observations regarding fat formation and deposition were confirmed by McHenry in 1937 (26), who also suggested that the effect of thiamin on fat synthesis could be harmonized with observations on the effect of the vitamin upon pyruvate utilization (36). In 1938, McHenry and Gavin began a series of reports on the relation of B vitamins to fat synthesis and metabolism. Thiamin was also found to cause synthesis in pigeons (37). Riboflavin and rice polish concentrate, used as a source of the B complex, given with thiamin, augmented the amount of synthesized fat (38). Pyridoxin, supplied with thiamin and riboflavin, likewise had a supplementary effect (39). In studying the effects of other members of the complex, an extract of beef liver was used as a source of unknown factors; this markedly augmented the amount of fat and caused fatty livers containing increased amounts of cholesterol (40). These fatty livers were not prevented by choline but were prevented by lipocaic (41). Later it was shown that biotin had a similar effect to that of the liver fraction (42) and that inositol acted like lipocaic in preventing the fatty livers caused by biotin in rats (43).

Included in the data arising from work with biotin, McHenry and Gavin gave results (42) which showed a specific effect of thiamin upon fat synthesis but to which they failed to call attention. The amounts of fat produced by various combinations of known B vitamins were cited. Riboflavin, pyridoxin, and pantothenic acid had augmentory effects upon fat synthesis. Examination of the data shows that biotin, while causing fatty livers, actually did not increase the percentage of body fat (as compared with the amount of body fat produced by all of the isolated B vitamins except biotin). The data show that rats given biotin were heavier and contained a larger total weight of fat but that the percentage of body fat was smaller. It is also clear from the data that no synthesis of fat occurred when thiamin was omitted from the supplements, even though five other B vitamins were supplied. The results obviously indicated that thiamin is essential for the synthesis of fat from carbohydrate and that other members of the B complex can augment the amount of synthesis.

Longenecker, *et al.* (44) studied the composition of the fats synthesized by the action of thiamin. Rats were depleted for three weeks on a diet free of B vitamins and of fat. At the end of this period one half of the number of animals were killed and tissue analyses made; the remaining rats were continued on the basal diet plus thiamin for twelve days, when they were killed and used for analysis. During the depleting period, body stores of fat were utilized and apparently no synthesis occurred; there was a marked increase in the iodine number of both liver and body fats. Thiamin administration caused a large increase in the amount of fat and a marked drop in iodine number; liver and body fats of supplemented animals contained a high proportion of $C_{16}$ acids, a finding which Longenecker had previously shown (45) to be characteristic of synthesized fat. In a later study the composition of liver and body fat as effected by other B vitamins, by liver fraction (as a source of biotin), and by lipocaic was investigated (46). Fatty acids synthesized when thiamin, riboflavin, pyridoxin, and choline were given were largely $C_{16}$ and $C_{18}$ acids, the $C_{16}$ acids being 54% of the total. Further supplementing the diet with the liver fraction caused a greater increase of $C_{18}$ acids than of $C_{16}$ acids and also augmented the quantity of unsaturated acids. This change in the fatty acid composition, and particularly the increase in unsaturated acids is of interest in connection with the question of essential fatty acids; in most of the studies on the latter subject, yeast has been used as a source of the B vitamins. It may well be that a concentration of the vitamins, different from that supplied by yeast, might cause a synthesis of essential fatty acids.

The hypothesis that thiamin has a specific effect upon fat synthesis has been criticized by Quackenbush, Steenbock, and Platz (47). Data from two types of experiments were cited in support of the criticism. In the first case, three groups of rats were used, all of which had been depleted on a diet containing autoclaved yeast, the first group was killed and analyzed at the end of the depleting period, the second group received thiamin and the basal diet *ad libitum*, the third group were given the same amount of thiamin as used in the second group but the intake of the basal diet was so restricted that body weights were held stationary. At