The Role of Vitamins in the Anabolism of Fats

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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 vita-

mins 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

the end of the depleting period, there was a marked reduction in the amount of fat and an increase in the iodine number. The second group showed a large increase in body fat and in body weight while the third group evidenced no increase in fat. Obviously, if food intake is restricted so as to maintain a constant body weight, there could be no increase in body fat unless other constituents, *e.g.*, protein or water, were diminished simultaneously. Restrictions of food intake on this basis would, of course, prevent any evidence of fat synthesis as shown by an increase in body fat. These results should be contrasted with those obtained by the use of isocaloric feeding. There have been repeated demonstrations of the specific effect of thiamin upon body weight and body fat when isocaloric feeding was used (26, 29). In such experiments the effect of thiamin upon metabolism is clearly evident. In the second type of experiment Quackenbush, *et al.* used a basal diet containing thiamin and riboflavin but designed to produce acrodynia. After maintenance on this diet for five weeks, one group of rats was killed for analysis, a second received extra thiamin for three weeks, a third was given a rice bran concentrate, and a fourth, with the same amount of rice bran concentrate, had the intake of the basal diet restricted so that a constant body weight was maintained; after these supplements had been given for three weeks the animals were killed for analysis. The first group showed only a small amount of body fat, and the authors interpreted this result as indicating that a continuous supply of thiamin had not prevented a loss in body weight nor accomplished fat formation. However, the iodine numbers of the liver and body fats were significantly lower than was the case in the group of animals not given thiamin in the first experiment, suggesting that fat synthesis had taken place. It is possible that, in the acute stages of pyridoxin and pantothenic acid deficiencies, fat is rapidly utilized and disappears soon after formation. Deficiencies of these vitamins were certainly more severe than had been obtained in the work of Gavin and McHenry (42). The question of the extent of deficiencies of these two vitamins was raised by Quackenbush, *et al.*; Gavin and McHenry had produced deficiencies of these vitamins to such an extent that animals responded readily to supplements but the criticism of this aspect appears to be partially justified. The third group of animals, receiving rice bran concentrate, showed a marked increase in fat. Quackenbush, *et al.* stated that this showed that a correction of any deficiency would bring about fat synthesis. However, they did not try the effects of pyridoxin nor of pantothenic acid in the absence of thiamin; under this condition it has been shown that fat formation does not occur (42). Indeed, a supply of four B vitamins to deficient animals will not cause fat synthesis *unless* thiamin is furnished (42). The additive effect of other B vitamins had been shown, previously to the

work of Quackenbush, *et al.*, by Gavin and McHenry in connection with riboflavin (38), rice bran concentrate (38), pyridoxin (39), pantothenic acid (42), and biotin (42).

In studies on the relation of the B vitamins to fat synthesis and metabolism the effects of lipotropic agents must be considered. For example, if thiamin is given as the only supplement to a basal diet rich in carbohydrate and low in protein, there will be a marked increase in liver fat (26). This increase can be prevented by supplying choline or a choline precursor, such as a large amount of casein. Choline can be inadvertently furnished. In the investigation of Engel and Phillips (48) fatty livers were obtained, but not acutely so because the basal diet contained peanuts and yeast, both good sources of choline. Quackenbush, Steenbock, and Platz (47) noted that fatty livers were not secured in their animals, even with thiamin supplementation. However, both of their experimental diets provided 18% casein, an amount sufficient to be lipotropic, and one of the diets contained two sources of choline: autoclaved yeast and starch.

In a recent paper, Stetten and Grail (49) have described studies on fat synthesis with the use of deuterium. The effects of pyridoxin and pantothentic acid in augmenting fat synthesis were confirmed, but these authors, like Quackenbush, *et al.*, did not give these vitamins without thiamin. As has been pointed out previously the addition of other B vitamins not only increases the amount of fat but also causes a change in the character of the fat. Reports by Engel (50), by Forbes (51) and by Gavin, *et al.* (52) have shown that the supply of various B vitamins alters the lipotropic effect of choline. This may partially explain the observation by Stetten and Grail regarding the very slight effect of choline in their experiments.

Longenecker, *et al.* (44) found that thiamin, as the only supplement, caused a marked synthesis of saturated C_{16} acids. If the diet is deficient in choline, or its precursors, fats accumulate in the liver; this can be prevented by a supply of choline. McHenry and Gavin (42) reported the production of fatty livers, by adding biotin and other B vitamins to the diet, which were resistant to the action of choline. Engel (50) has found that thiamin, riboflavin, pyridoxin, and pantothenic acid produce a choline-resistant type of fatty liver. Under this condition choline and inositol, given simultaneously, will maintain liver fat at a normal level. Engel believed that pyridoxin was concerned with this effect since small amounts of choline were effective in animals depleted of pyridoxin for eight weeks or longer. A different result was obtained by Forbes (51), who found that choline was less effective in rats receiving nicotinic acid. These findings of Engel and of Forbes again raise the point regarding alterations in the type of fatty acids synthesized by changes in the B vitamin supplements. This deserves further study. When thiamin is the only B vitamin supplied, the character

of the fat synthesized and deposited in the body is radically different from that found in "normal" animals. The question as to which B vitamins should be given to animals to cause the production of a more "normal" type of body fat is a matter for further investigation. It may well be that the quantitative proportion of the B vitamins may be a factor, influencing the character of the synthesized fat.

Some attention has been given to investigation of possible effects of other dietary factors in fatty acid synthesis or retention. Sheppard and McHenry (53) reported that the fat content of vitamin-C-deficient guinea pigs was greatly reduced in comparison with that of normal animals and that pair-fed guinea pigs receiving ascorbic acid contained even less fat than the members of the deficient group. In these experiments, ascorbic acid did not apparently cause fat synthesis. However, experiments carried out by Patterson, McHenry, and Crandall (54) indicated an effect of vitamin A upon either fat synthesis or retention. Using a diet which provided only a very small amount of lipid material, pair-fed rats receiving carotene contained more fat than did the deficient control animals and practically as much as rats fed *ad libitum*. These results were in agreement with observations reported in 1932 by Sampson and Korenchevsky (55). These authors had also noted the higher fat content of pair-fed rats receiving vitamin A. It is not clear as to whether the increase in fat content was due to synthesis and further investigations are needed.

IV. The Synthesis of Fat from Protein

After reviewing evidence regarding the formation of fat from protein, Mitchell and Hamilton (56) stated in 1929, "of all the possible transformations of nutrients in the animal body, that of the conversion of protein into fat, or, more correctly, of amino acids into the higher fatty acids entering into the composition of animal fats, has probably attracted the greatest amount of experimental enquiry and occasioned the most controversy. . . . Purely on chemical grounds, the conversion of protein to sugar to fat, involving a complicated and uneven series of cleavages, oxidations, and condensations, would seem to be a clumsy and thoroughly inefficient method of disposing of an excess of dietary protein." After considering the demonstrations of fat synthesis from protein in bacteria by Beebe and Buxton (13), and in mammalian eggs by McClendon (57), Mitchell and Hamilton concluded that, while a clear-cut proof of fat formation from protein had not yet been provided in higher animals, a considerable probability that such a transformation does occur under certain conditions had been established.

While the work of Lawes and Gilbert (7) is generally cited (2) as the first proof of fat formation from carbohydrate, a careful examination of their data shows that a reasonable amount of the fat synthesis which they ob-

served must have been obtained from protein. The first completely convincing evidence of the ability of higher animals to convert protein to fat was furnished by Longenecker in 1939 (22) and confirmed by Hoagland and Snider in the same year (23). In both cases rats were maintained on diets rich in casein but free of fat and carbohydrate. On such a diet Hoagland and Snider secured a gain in body weight of 67% and an increase of body fat of 257%. The procedure used by Longenecker is of interest because it has been used by other workers and has been criticized. A loss in body weight of about 25% was produced in rats by inanition, and the animals were then fed a special diet until weight was restored. The special diet used to demonstrate the synthesis of fat from protein contained 96% casein, was free from carbohydrate or fat, and B vitamins were supplied in yeast. It was found that large amounts of fat were synthesized from protein and that, consequently, the body weights of the rats were increased. Longenecker gave data regarding the composition and characteristics of the synthesized fat. While the inference was drawn that fat from protein was quite similar to that obtained from carbohydrate, the data show some differences. These are worth noting because it would be assumed that, if fat is formed from protein through an intermediate carbohydrate stage, this fat should be similar to that synthesized from carbohydrate supplied in the diet. "Protein-fat" contained a larger proportion of saturated fatty acids than did "carbohydrate-fat"; in terms of some individual fatty acids, "protein-fat" contained more palmitic and arachidic acids and less oleic acid. The conclusion that "protein-fat" is entirely similar to "carbohydrate-fat" was drawn by Eckstein in 1929 (58). In this connection reference should again be made to the statement by Mitchell and Hamilton that it is unlikely that the synthesis of fat from protein proceeds by way of carbohydrate. However, the character of the fat synthesized from either carbohydrate or protein may depend upon the vitamin supplements.

At least three types of evidence have indicated that one or other members of the vitamin B complex are concerned with protein metabolism. In 1927, Hasson and Drummond (59), and in 1928, Hartwell (60) showed that an increased supply of the B vitamins was necessary for normal increase in weight when high-protein diets were fed; Hasson and Drummond indicated that two factors, one of which was heat-stable, were essential for protein metabolism. Investigations by Richter and Hawkes in 1940–1941 (61, 62) on the choice of foodstuffs by rats depleted of several members of the B complex also indicated a relationship between proteins and these vitamins.

In 1940, McHenry and Gavin (63), using a procedure patterned after that of Longenecker, studied the effects of various B vitamins upon the synthesis of fat from protein. Adult rats, about 200 g. in weight, were placed for three weeks upon a diet free of fat and of the B vitamins. During

this depleting period the amount of body fat was sharply reduced, and there was a loss in body weight of about 25%. After this period the animals were fed a diet containing 96% casein but no fat nor carbohydrate; B vitamins were given singly and in various combinations. This treatment was continued for 10 days when the rats were killed and fat determinations made. Only in those animals which received thiamin and pyridoxin was there any evidence of fat formation and consequent increase in body weight. When thiamin, riboflavin, pantothenic acid, nicotinic acid, and choline were all given, the loss in body fat and body weight, characteristic of the depleting period, was continued throughout the supplemental period. In contrast, animals which received the same supplements plus pyridoxin showed a three-fold increase in body fat. On the basis of these observations it was concluded that pyridoxin is concerned with protein utilization.

In an extension of these observations Cornett and McHenry (unpublished data) have shown that pyridoxin and thiamin are also essential for the formation of glycogen from protein. They found that the addition of biotin to the supplements, which, in the case of high carbohydrate diets, quickly causes the production of fatty livers containing large amounts of cholesterol, fails to do so with protein-rich diets. These preliminary results suggest that the path for the synthesis of fat from protein does not proceed by way of carbohydrate; otherwise, one would expect a similar result to that secured with a carbohydrate diet.

A vigorous criticism of this recent work on the relationship between the B vitamins and fat synthesis from protein has been made by Mitchell (64) and repeated by him more recently (5). After an incomplete description of the procedure employed by McHenry and Gavin, a description which omitted a fundamental feature, Mitchell stated:

"Only when pyridoxin was fed was the fatty acid content of the carcass appreciably greater than that of the controls, but only in such cases did any growth occur. However, fat deposition is a necessary concomitant of growth and, regardless of the nature of the dietary characteristics that induce variable intakes of food, there will in general be a close correlation between the rate of growth secured and the fat content of the carcasses produced, within the range of submaximal rates of growth. For this reason, for instance, the supplementation of a ration deficient in sodium or chlorine by sodium chloride might be expected to produce, not only more rapid gain in growing rats, but also greater contents of carcass fat, without any implication that either sodium or chlorine is at all intimately involved in fat synthesis. Such technic cannot be expected to yield evidence of a direct and fundamental relationship between any dietary supplement and the synthesis of body fat."

It may be assumed that the essential nature of Mitchell's criticism is that

rats on a deficient diet do not grow as well as animals on an adequate diet and that the latter rats, because of better growth, will always contain more fat. This viewpoint is based, when used as a criticism of the results of McHenry and Gavin, upon an erroneous and misleading conception which is unfortunately evident in many papers on animal nutrition. For some years it has been the custom to plot animal weights and to assume that the result is a "growth" curve. Many dietary constituents, particularly the vitamins, have consequently been described as essential for growth. The fallacy of this misuse of the word "growth" was clearly pointed out by Orr and Richards in 1934 (65) but the fundamental point raised by them has been completely ignored by many investigators in the field of nutrition. Orr and Richards showed that, in rats deprived of vitamin A, the body weight reaches a maximum and then decreases, but true growth as measured by body length actually continues. As "growth" is interpreted in the current literature, rats in acute vitamin A deficiency should be described as exhibiting negative growth, a description which would be patently absurd but no more fallacious than the use of body weight as an index of growth. Fortunately, this misconception has not been used with humans; if it were, there would be the interesting illustration of a man showing two, or perhaps more, periods of growth during life, one during childhood and adolescence, and another in middle age. The use of weight as an index of growth in animal experiments is not only misleading and inaccurate but has actually handicapped advance in knowledge of the relation of the vitamins to metabolism. The time has surely arrived when workers in nutrition should use the simple word growth correctly.

In the case of the investigation criticized by Mitchell, emphasis on the misuse of the word growth is particularly pertinent. Adult rats were depleted for three weeks, during which time there was a marked loss in weight (but not negative growth). The effects of various supplements were then tried with the results reported above. Because there was a synthesis and deposition of fat, and perhaps of other substances, the body weight was, of course, increased. Since body length was not measured it is impossible to say whether growth also occurred. It does not always follow, as Mitchell assumed, that an increase of fat is concomitant with an increase in body weight. Sheppard and McHenry reported in 1939 (53) that guinea pigs receiving ascorbic acid but isocalorically fed with deficient controls are significantly heavier but contain much less fat than the deficient animals. In pointing out that any dietary supplement, by increasing weight, would also increase body fat, Mitchell used as an example either sodium or chlorine. In 1937 Kahlenberg, Black, and Forbes (66) reported that sodium supplementation enabled rats to synthesize fat. This was concluded from results of fat analysis of isocalorically fed animals. Unfortunately, no clear

conclusion as to fat synthesis was possible since the basal diets contained variable amounts of fat and the difference in fat intake between the two groups more than accounted for the difference in fat deposition.

A number of studies of body composition of rats of different ages have been made but in each case growth has been determined by body weight. An interesting study of the data from one investigation can be made. Pickens, Anderson, and Smith (67) reported the results of analyses of rat bodies at different ages. It has seemed advantageous to combine their data with figures for body length given by Donaldson (68). At an age of 100 to 120 days, curves for total ash, for protein, and for body length show plateaus while body weight and body fat continue to increase. Apparently, at that age, true growth ceases but the weight continues to be increased by a "fattening" process. A failure to distinguish between growth and changes in body weight and an interpretation that animals became heavier simply because of some mysterious growth process diverted the attention of investigators from fundamental studies on metabolism and delayed progress in the vitamin field. A good example of this was the important and useful report by Mitchell in 1933 (34) regarding the effect of vitamin B in causing a weight difference between rats fed isocalorically. Data were available to focus attention upon the relation of thiamin to fat synthesis but, unfortunately, this was not done until the question was examined by Whipple and Church three years later (29).

Additional evidence that pyridoxin is concerned with protein metabolism was supplied by Foy and Cerecedo (69), who showed that a deficiency of this vitamin is much more rapidly produced in rats by maintaining them upon a protein-rich diet. Pyridoxin has not yet been linked to a particular enzyme system and further investigations will be needed to elucidate the rôle played by pyridoxin in protein degradation.

V. The Formation of Phospholipids

So far as can be ascertained, no effect of vitamins upon the synthesis of phospholipids has been described, unless choline is considered to be a vitamin. While such a classification of choline seems to be unnecessary, a number of writers have done so, and a brief reference to this substance should be included. For a detailed discussion of choline, recent reviews by McHenry (70), by Griffith (71) and by Best and Lucas (3) may be consulted. The anabolism and function of the phospholipids has recently been reviewed by Sinclair (72).

The demonstration that choline would prevent the formation of some types of fatty livers led to several explanations of its lipotropic action. Of these, the most plausible one, for which evidence was first provided by Welch (73), is that choline is lipotropic because it promotes the formation

of phospholipids. Perlman and Chaikoff (74) have furnished confirmatory evidence for this assumption. Choline is most effective as a lipotropic agent when fat accumulation in the liver consists mainly of simple glycerides and, more particularly, when the fatty acids are largely C_{16} or C_{18}, with a low iodine number. The activity of choline is influenced by several B vitamins (54); this may be due to a change in the character of the fatty acids present in the liver. The lipotropic effect of choline is also inhibited by the presence of large amounts of cholesterol (54). It may be that phospholipids can only be formed by the action of choline from certain fatty acids and not from an indiscriminate assortment.

Inositol has been shown by Gavin and McHenry (43) and by Engel (50) to have a lipotropic action. Inositol is present in considerable amounts in some naturally occurring phospholipids (75), and it is possible that inositol, like choline, owes its lipotropic activity to participation in the formation of phospholipids. There is no direct experimental evidence for the correctness of this hypothesis. It is of interest that inositol exerts its influence under conditions unfavorable for the action of choline, particularly in the presence of large amounts of cholesterol. Analysis of the phospholipids formed by the action of choline and of inositol would provide useful information regarding the mode of action of these two substances.

VI. The Synthesis of Cholesterol

The ability of the animal body to synthesize cholesterol was clearly shown by Channon (76), by Randles and Knudson (77), by Eckstein and Treadwell (78), and by Schoenheimer and Breusch (79). Schoenheimer and Breusch showed that mice maintained on a diet of bread, or of bread and fat, synthesized as much cholesterol in a month as was present in their bodies at the start of the experiment. Their work indicated that there is continuous formation and destruction of cholesterol and also that the administration of large amounts of fat has no significant effect upon cholesterol synthesis.

The nature of the metabolic precursors of cholesterol has been somewhat uncertain. Eckstein and Treadwell (78) believed that the use of a high fat diet caused increased formation of cholesterol. Rittenberg and Schoenheimer (80) concluded that cholesterol is formed by the coupling of a large number of small molecules. Their observations failed to support a possible conversion of fatty acids to sterol directly, but they stated that their results could be explained by the theory that cholesterol plays a rôle in the transport of fatty acids in the organism and that the handling of a larger amount of fatty acids may require the presence of a larger amount of cholesterol, the latter being formed according to the need.

In 1937, Sonderhoff and Thomas (81) reported that yeast, grown in a

medium containing deuteroacetate, had so great a deuterium content in the unsaponifiable portion that it seemed necessary to postulate the conversion of acetic acid to sterols. Bloch and Schoenheimer (82) and Bloch and Rittenberg (83), also using deuteroacetic acid, demonstrated that mice are able to synthesize cholesterol from acetate. This confirmed the previous report from Schoenheimer's laboratory that cholesterol is formed by the union of a number of small molecules (80).

In 1940, McHenry and Gavin (40, 41) reported that the feeding of a beef liver fraction, used as a source of unisolated components of the vitamin B complex, caused a marked increase in liver and body cholesterol in rats. It was stated that this increase was due to synthesis, since the basal diet was practically devoid of cholesterol. This observation was confirmed by Longenecker, *et al.* (46). Gavin and McHenry (42) later found that an effect, similar to that produced by the liver fraction, could be obtained by the administration of biotin in conjunction with thiamin, riboflavin, pyridoxin, and pantothenic acid. However, they pointed out that biotin did not increase the amount of cholesterol synthesis but did augment the amount of cholesterol in the liver. The increase in liver cholesterol is either the cause of, or accompanies, the production of a type of fatty liver which is not prevented by choline but which is prevented by lipocaic or by inositol. The data showed that the increase in liver cholesterol did not precede, but paralleled, the increase in liver fat. Results, subsequently reported by Gavin, Patterson, and McHenry (52), showed that the increase in liver cholesterol caused by biotin is either due to, or is coincidental with, a slight decrease in body cholesterol. It would appear that biotin causes an accumulation of cholesterol in the liver, perhaps at the expense of the amount in the body; the increase in liver cholesterol is prevented by lipocaic or by inositol. All three dietary supplements would seem to have effects upon cholesterol transport.

In a study of the influence of various B vitamins upon the production of fatty livers, Forbes (51) has secured somewhat different results. He found that the administration of nicotinic acid to rats caused an increase in liver cholesterol, in comparison to that obtained with other B vitamins but without nicotinic acid.

Unpublished observations by Gavin and McHenry on the influence of various B vitamins upon cholesterol synthesis indicate that the total amount of cholesterol approximately parallels the total amount of fat. It should be pointed out that these experiments were carried out with a high-carbohydrate, low-protein, fat-free diet, and presumably the cholesterol was synthesized, like fat, from carbohydrate. When thiamin was not supplied to the animals there was no evidence of either fat or cholesterol synthesis. These observations, and those which have previously been mentioned,

do not supply proof that any of the B vitamins are directly concerned with the formation of cholesterol. The data are consonant with the theory, expressed by many writers over a period of years, that cholesterol formation keeps pace with the amount of fat present in the body.

VII. Possible Mechanism of Fatty Acid Synthesis and the Relation of Vitamins to Enzyme Systems

Although it was proven many years ago that animals can synthesize fats, and although this synthesis must constitute a prominent phase of metabolism, the chemical changes involved in such interconversions remain quite obscure. Several hypotheses have been advanced but in the main they have been based on either chemical information derived from *in vitro* experiments or upon biochemical observations on microorganisms. The applicability of these hypotheses to higher animals is a matter of doubt. No attempt will be made to review the theories in detail but some mention is desirable in order that a possible relationship of the B vitamins to fat synthesis may be considered.

One of the early theories regarding fat formation was that postulated by Emil Fischer (84). He believed that fatty acids could be formed from sugar molecules by direct condensation and subsequent reduction of the condensation product. For example, stearic acid would be formed from three hexose units, and palmitic acid would then be derived from stearic acid by β-oxidation. The prevalence in nature of fatty acids possessing structures which are multiples of 6 carbons favors the theory. Chemical evidence is against the acceptance of the theory, and no biochemical evidence in support of it has been forthcoming in recent years.

The importance of acetaldehyde, derived from carbohydrate, as a possible intermediate in the formation of fatty acids was emphasized by Nencki (85), by Hoppe-Seyler (85), and by Magnus-Levy (85). The theory which was developed on this basis was somewhat as follows: Units of acetaldehyde, formed by breakdown of lactic acid, underwent aldol condensation. From the aldol there were two possible products, either butyric acid or croton aldehyde which would finally be converted into butyric acid. Long chain fatty acids could be obtained by repeated condensations of aldehydes. An objection to this theory, that the condensation of aliphatic aldehydes with acetaldehyde usually yields a product with a branched-chain structure, has been found not to hold for the autocondensation of aldol (86). Aldol can be formed by mild chemical treatment of acetaldehyde (85), and the ability of the liver to effect aldol condensations has been reported (87). Evidence secured by the use of microorganisms has both supported and denied this theory. Fitz (84) was unable to demonstrate the presence of acetaldehyde or aldol in the lactate medium in which bacteria were producing butyric

acid, and Smedley-MacLean (84) reported similar observations. While studies on yeast have shown that acetaldehyde is formed during the degradation of sugar, they have failed to provide evidence as to the importance of acetaldehyde in some cases but, in others, have shown that acetaldehyde can be used in the formation of fatty acids (88, 89).

Another possible theory has been suggested by Smedley-MacLean. In this hypothesis considerable importance is given to pyruvic acid as the intermediate between carbohydrate and fat. The initial stages in the formation of fatty acids were stated by Smedley-MacLean (84) to be as follows:

$$CH_3\cdot CO\cdot COOH + CH_3\cdot CO\cdot COOH$$

(pyruvic acid)

$$\longrightarrow CH_3\cdot \overset{\overset{COOH}{|}}{\underset{\underset{OH}{|}}{C}}\cdot CH_2\cdot CO\cdot COOH$$

(α-keto-γ-hydroxy-γ-carboxy-valeric acid)

$$\longrightarrow CH_3\cdot \underset{\underset{OH}{|}}{CH}\cdot CH_2\cdot CO\cdot COOH + CO_2$$

$$\longrightarrow CH_3\cdot CH{:}CH\cdot CO\cdot COOH + H_2O + CO_2$$

$$\longrightarrow CH_3\cdot CH{:}CH\cdot CHO + H_2O + 2CO_2$$

(crotonaldehyde)

It was suggested that the crotonaldehyde, which had thus been produced, might condense with another pyruvic acid molecule to yield a higher keto acid, capable of either oxidation or further condensation. This scheme would permit the formation of highly unsaturated aldehydes which could be converted into fatty acids.

Support for the hypothesis of Smedley-MacLean is furnished by several entirely different types of experimental evidence, of which the following may be cited: Unsaturated aldehydes have been identified in aerated sugar solutions in which yeast has been cultured and in which the fat content is increased (90). Yeast cells will cause the production of butyric acid and carbon dioxide from the lactone of α-keto-γ-hydroxy-γ-carboxy-valeric acid, the compound which is the first condensation product in the Smedley-MacLean sequence (84). Smythe (91) has reported that yeast cells will utilize pyruvic acid under aerobic conditions, that the respiratory quotient is increased above unity, and that the amount of ether-soluble material in the culture is increased. Smythe found that this effect could be secured with pyruvic acid in the media but that it failed to occur when acetaldehyde was substituted for the pyruvic acid.

In contrast to the results obtained by Smythe, it has been reported by

Smedley-MacLean and Hoffert (92) that yeast will bring about lipid formation from acetic acid or ethyl alcohol. Sonderhoff and Thomas (81) have suggested that fatty acids can be synthesized from the acetate ion; MacLeod and Smedley-MacLean (93) have reported observations which are also indicative of this process. Krebs and Johnson (94) have shown that animal tissues can form butyric acid from acetaldehyde. Investigations of the fates of labelled fatty acids in mice by Stetten and Schoenheimer (95) have provided information regarding fatty acid metabolism; they observed that fatty acids could undergo desaturation and also the loss of 2 carbon atoms, and that units of this size could be added to fatty acid chains. Stetten and Schoenheimer (95) suggested that the condensation by which the union would be affected would require a reactive terminal group as would be supplied by an aldehyde. While there is evidence to support the Smedley-MacLean theory, there are also data tending to confirm the previously advanced hypothesis that acetaldehyde is the likely intermediate between carbohydrate and fat. It might be suggested that pyruvic acid is the true intermediary, since acetaldehyde would likely be formed from pyruvic acid.

It is useful to consider how the theories regarding fat synthesis can be reconciled with the observations that thiamin, and perhaps other B vitamins, are essential for fat synthesis. The rôle of thiamin in this process is easier to understand than is the parts played by the other B vitamins. It is now well established that thiamin diphosphate functions as cocarboxylase in yeast (96) and, in the same form, is necessary for the enzymic disposal of pyruvic acid in more highly specialized tissues (97). Emphasis has been placed by Peters (97) upon the rôle of thiamin in the production of energy from carbohydrate. It may be that it is also needed for the production of compounds participating in the condensation reactions necessary for the synthesis of fatty acids. It is established that pyruvic acid accumulates in the tissues of thiamin-deficient animals, that a supply of the vitamin will cause a marked reduction in the amount of pyruvic acid (97), and that there is fatty acid synthesis (29). A simple explanation would be that thiamin causes the production of acetaldehyde from pyruvic acid and thus initiates the sequence of reactions which brings about the synthesis of fatty acids. This would harmonize the known information about the function of thiamin with the theory regarding acetaldehyde being the intermediate between carbohydrate and fat. It should also be pointed out that the Smedley-MacLean hypothesis requires decarboxylations, a type of reaction in which thiamin could function. It seems to be not unreasonable to suggest that the future work on the mechanism of formation of fats should include the accredited information about the function of thiamin.

While the probable rôle of thiamin in fat synthesis can be suggested, the functions of other B vitamins in this process are quite indefinite. The ex-

periments of McHenry and Gavin (38, 39, 41, 42, 43) showed that other members of the B complex, given in conjunction with thiamin will augment the amount of fat synthesis. In confirmation of this observation Quackenbush, *et al.* (47), Engel (50), and Stetten and Grail (49) have shown that pyridoxin and pantothenic acid increase the quantity of synthesized fat. While coenzyme functions have been described for riboflavin, nicotinic acid, and biotin, and suggested for pyridoxin and pantothenic acid, their rôles in fatty acid synthesis are obscure.

Discussions of the mechanism of fat synthesis have tended to assume that there is one condensation pattern by which fatty acids are built up in plants and in animals. This may be true, but there is one marked difference between fat synthesis in plants and in animals. Some plant oils contain large amounts of linoleic and linolenic acids, compounds which apparently cannot be synthesized under the conditions which have been employed in experiments on rats. It has been pointed out previously in this review that there is evidence suggesting that the character of the synthesized fat can be altered by variations in the supplements of the B vitamins. It is interesting to speculate whether differences between plant and animal fats are due, perhaps in part, to variations in the B vitamins which are present. Determinations of the amounts of the different B vitamins contained in ripening seeds might provide useful information upon which to design animal experiments for a study of the possible production of essential fatty acids.

VIII. Thiamin-sparing Action of Fats

Derangement of carbohydrate metabolism in thiamin deficiency, manifestations of which include elevation of blood sugar, pyruvate, and lactate, led to the early recognition of the essential nature of this factor in the catabolism of carbohydrate (98, 99). The necessity for inclusion of large amounts of carbohydrate in thiamin-deficient diets in order to produce deficiency symptoms in animals (100, 101) and the capacity for fat to "spare" the vitamin has been demonstrated by several groups since the relationship was originally pointed out by Evans and Lepovsky (102, 103) in 1928–1929. These workers reported continued growth and a failure in appearance of the typical deficiency syndrome in rats when the "antineuritic-vitamin-free" diet contained 50% lard. The animals were observed for a period of six months. Subsequently, Evans and Lepovsky (104) pointed out that even on a high carbohydrate diet containing presumably an adequate amount of yeast as well as a source of the unsaturated fatty acids, animals exhibited a favorable response to the provision of additional fat. Comparison of the sparing-effects of several natural fats and oils indicated that neither the precise melting-point nor the degree of saturation influenced the activity. However, the melting-point had to be sufficiently

low to permit good absorption (105). These experiments were extended to include a study of the activities of glycerides of single fatty acids (106). Because of poor absorption, stearin was ineffective; myristin and caprylin exhibited the greatest potency. Subsequent observations reported by Salmon and Goodman (107) are in harmony with these results. They compared the thiamin-sparing capacities of various pure fatty acid esters and noted that fats having an 8-carbon structure exhibited the maximum potency. Shorter chain acids possessed toxic properties while the higher molecular weight members of the series were decreasingly active.

The sparing action of fat on thiamin requirements has been widely confirmed. Guerrant and Dutcher (108) also reported this effect, and Whipple and Church (29) described similar observations. Salmon and his associates (109, 110) have carried out comprehensive studies on this phase of the relationship of fat metabolism to vitamin activity.

In most of these investigations, the extent of change in body weight and the degree of polyneuritis were the criteria of the "thiamin state" of the animals. However, MacDonald and McHenry (111) have described the effect of fat in delaying the onset of the characteristic bradycardia of thiamin deficiency in the rat. Banerji (112) confirmed this finding and also contributed the observation that inclusion of various amounts of fat in thiamin-deficient diets proportionally counteracts the augmented excretion of bisulfite-binding substances which is typical of the syndrome.

Explanation of the mechanism of this sparing action of fat has proved to be a provocative subject. Two possible hypotheses were eliminated; Evans and Lepovsky (113) demonstrated that the activity was not attributable to any vitamin-fat complex formed in the gastro-intestinal tract, and Melnick and Field (114) showed that thiamin was absent from the fats which gave a sparing action.

Because of the typical failure in carbohydrate utilization and the associated accumulation of pyruvic acid in thiamin deficiency, the fat effect has often been interpreted as resulting from the substitution of lipid for carbohydrate as a source of energy with a consequent decrease in the need for thiamin. However, evidence indicative of fatty acid synthesis as a function of thiamin has been reported (29) and the exclusion of this observation from any explanation of the sparing effect of fat is hardly justified. For many years, deposited fat was regarded as existing in a relatively static state. By means of tracer experiments the dynamic condition of not only the phosphorus-containing lipids of kidney and liver but also of the fat depot glycerides themselves has been demonstrated. Schoenheimer and Rittenberg (20), to whom a great deal of the credit for clarification of this concept is due, have expressed the situation as follows: "The results show that the fat depots represent a much more active organ than has been thought hitherto.

. . . Mice, like almost all other animals, take food only at intervals; the absorbed constituents of the diet are not burned immediately but must be deposited for short periods. Part of the carbohydrate (and carbohydrate precursors) is deposited as glycogen and is always available for combustion directly in the form of carbohydrate. However, the amount of glycogen which may be stored in the organs is relatively small. Most of the absorbed carbohydrate is therefore immediately transformed into fatty acids. These are deposited in the fat depots and utilized for combustion in the postabsorptive periods." This viewpoint places a new emphasis upon the importance of fat in animal metabolism. Over a period of some years great importance has been given to carbohydrate as the prime source of energy. While carbohydrate may be the source of energy for muscular work, it is possible that fat is the fuel for basal metabolism. The body would then have a definite need for fat, a requirement which could be satisfied by the inclusion of fat in the diet or by the conversion of carbohydrate to fat under the influence of thiamin and perhaps other B vitamins. On this basis there would be a requirement for thiamin to bring about the synthesis of fat, a requirement which would be lessened by the presence of fat in the food supply.

IX. Essential Fatty Acids

In 1929, McAmis, Anderson, and Mendel (115) reported the superior growth of rats provided with a small amount of fat in comparison with animals not receiving fat. In the same year, Burr and Burr (116) described a characteristic syndrome which appeared in rats fed a diet of ether-extracted casein, fat-free yeast, sucrose and salts. Under the dietary conditions described, the animals continued to increase in weight for some time but plateaued prematurely and finally, in the acute stages of the deficiency, lost weight rather rapidly. In consideration of these observations Burr and Burr discarded the explanation that the supply of a yeast factor was inadequate. They pointed out: "Whether the effect is caused by the strain of long-continued fat synthesis, suggested by Krogh and Lindhard, or whether a special type of fatty acid is required by the animal which it is unable to synthesize from the diet consumed, are unanswered questions." Relatively small amounts of lard and cod liver oil exhibited dramatic curative potencies, and the exceptional activity of liver in this regard was also indicated. Its effectiveness was superior to that of any of the fats. Subsequently Burr and Burr pointed out that the varying potencies of the fats were relatable to the structures of the component fatty acids, and the observation was made that in the animal deprived of fat the supply of an unsaturated fatty acid fraction of tissue lipids was depleted. The essential nature of dietary linoleic, linolenic, and possibly arachidonic acids was indicated (117).

Evans and Lepovsky contributed observations which are in accord with these results. They too suggested that factors other than relative inadequacy of vitamin B were concerned in the etiology of the disorders observed in animals fed strictly fat-free diets (118). The importance of the provision of unsaturated fatty acids was demonstrated by means of an experiment in which rats received a diet containing laurin as sole source of fat. Such animals exhibited even poorer growth than those receiving no fat at all (119). Sinclair, using a high elaidin diet has observed a similar relationship (120). Evans and Lepovsky also noted the depression in essential fatty acid content of the lipids of fat-starved rats (121).

The question of whether several acids were necessary or simply a key structure from which others could be formed became the subject of experimental work. Nunn and Smedley-MacLean (122) investigated the hepatic fatty acid structures of fat-deficient rats and described the absence of any acid containing 20 or 22 carbon atoms and 4 or more double bonds. Recent observations have indicated that linoleic acid, as well as being active *per se*, is used in the synthesis of arachidonic, and possibly clupanodonic acids (123, 124). Turpeinen (125) recognizing the superior curative capacity of arachidonic acid, suggested that it is of predominant importance. On the other hand, Burr, Brown, Kass, and Lundberg (126) have maintained that linoleic, linolenic, arachidonic, and cod liver oil fatty acids exhibit differences in both the qualitative and quantitative aspects of their actions. They expressed the view that, like the essential amino acids, these fatty acids should be treated as separate nutritional entities. However, in an excellent review of the subject, Burr (127) has recently pointed out that, strictly speaking, linoleic acid only is essential. It is of interest that MacKenzie, MacKenzie, and McCollum (123) reported the maintenance of rats in excellent condition for a year on a highly purified diet consisting of specially ether-extracted yeast, extracted casein, salts, and supplements of calciferol, carotene, cystine, sucrose, vitamin E, and methyl linoleate.

Burr and Burr, in an early publication (117), had suggested that the essential fatty acids were necessary for normal fat formation from non-lipid sources. Emaciated animals on the fat-free diets consumed as much food as the normal controls but apparently oxidized it, since growth and fat synthesis were not evident. Smedley-MacLean and Nunn (129) failed to confirm these observations and indicated that, although the capacity of the animal to store fat was impaired, the fatty-acid-synthesizing mechanism remained intact. Subsequently, however, these authors rejected this hypothesis and concluded that the polyunsaturated acids were concerned with neither the synthesis of fat nor its storage. Rats existing on the fat-deficient diet for long periods, contained actually a higher proportion of

lipids than controls which had received linseed oil. During the first six months on the fat-deficient diet the rats' supply of polyunsaturated fatty acids was reduced to a low level. During the second six month period, growth having ceased, the rate of utilization of these substances was markedly depressed. On the basis of observations such as these, the suggestion was advanced by Smedley-MacLean, *et al.* that these acids function in some as yet unelucidated way, in the formation of new cells. The growth of the Walker tumour in normal rats was associated with a decrease in the amount of subcutaneous fat and a depression in the proportion of highly unsaturated fatty acids (130). Whatever may be the functions of unsaturated fatty acids in the growth process they are probably involved also in fat transport. Engel (50) has shown that the lipotropic action of choline is evident only when linoleic acid is provided. Thus, both these phospholipid constituents must be available.

The limited information available on fatty acid deficiency in species other than the rat has been reviewed by Burr (127). Attention should be drawn to an interesting observation made on an adult human who existed for a six month period on a fat-free diet (132). The arachidonic and linoleic acid contents of the serum lipids were depressed and, in the post absorptive periods, active fat synthesis was indicated by respiratory quotients well above unity.

The essential fatty acid problem has been rendered more complex by experiments which indicated a possible relationship to the metabolism of the B vitamins. Attention having been drawn to the similarity between the acrodynia of pyridoxin deficiency and the dermal abnormalities of unsaturated fatty acid deficiency (133), observations published by Birch and György (134) suggested that fat exerted a sparing action on the metabolism of pyridoxin. Quackenbush, *et al.* (135) reported that the symptoms developed on a diet lacking pyridoxin could be alleviated by the administration of various natural fats and synthetic esters and the activity of the fats in this regard paralleled their degree of unsaturation. Linoleic acid was apparently capable of curing both the acrodynia and the essential fatty acid syndrome. The activity of the fat could not be attributed to pyridoxin contained in it. It was pointed out that the acrodynia could also be relieved by means of a rice bran concentrate (136).

Results published by Salmon, however, were not in harmony with these observations. He reported that both a heated yeast extract and certain oils were necessary for the maintenance of a normal skin condition (137), and Birch (138) also indicated that both fat and water-soluble factors were necessary. Subsequently Salmon showed that the dermatitis in rats provided with casein, sucrose, salts, carotene, calciferol, α-tocopherol, thiamin,

riboflavin, and choline could be cured completely only by the administration of pyridoxin, linoleic acid, and pantothenic acid (139).

Recently Quackenbush, Steenbock, Kummerow, and Platz (140) reviewed the situation and pointed out that the discrepancies among the results previously reported could be attributed to the use of diets of questionable lipid content and undefined sources of water-soluble factors. In rats given a highly purified, fat-free diet, curative tests showed that linoleic acid was the primary factor concerned. In sufficient amounts it was completely effective; in subcurative amounts pyridoxin enhanced the action of the fatty acid. Prophylactic tests indicated that pyridoxin was capable of retarding the development of the skin condition, and pyridoxin plus pantothenic acid were still more effective. In this publication it was pointed out that the "accessory factor" of rice bran concentrate reported by Schneider, *et al.* (136) to be necessary in addition to pyridoxin in the cure of acrodynia appeared to consist of pantothenic acid as well as some other substance. As stated by the authors, "whether the additional factor is a known dietary essential remains to be determined." Although a relationship of these several factors to the metabolism of certain fatty acids has been indicated there has been no suggestion as to the mechanism of action, nor, indeed, proof that such a relationship exists.

It seems odd that an organism possessing mechanisms which make possible the synthesis of structures such as oleic, stearic, and palmitic acids and capable of desaturating, hydrogenating, and adding carbon atoms to a fatty acid chain would be incapable of promoting such closely allied reactions as the formation of linoleic, arachidonic, and other acids. In view of the observation of Sinclair (120) that rats exhibited superior growth on a high carbohydrate, fat-free diet in comparison with those on a high-elaidin regimen, it is interesting to consider the two possible explanations presented by the author. He pointed out that either the ingestion of fat augmented the *requirement* for certain fatty acids, or that the inclusion of fat in the diet suppressed the process of *synthesis* of fat, including that of the unsaturated acids essential for growth.

The possibility thus remains that the animal body may be capable of synthesis of these acids when the necessary dietary constituents are provided. In much of the work outlined, yeast was used as the source of the B vitamins. Some attention has been drawn to the inadequacy of the diets employed in this respect, and an increase of the yeast intake has been tested for effects upon the syndrome (32, 33). It was usually concluded that the disorder could not be related to adequacy of any B factor. However, according to R. J. Williams (141) the vitamin content of brewers' yeast assayed as follows, by microbiological methods:

Factor	*γ per g.*
Thiamin	8.5
Riboflavin	15.2
Nicotinic acid	126.0
Pantothenic acid	42.5
Pyridoxin	1.0
Biotin	0.071
Inositol	280.0
Folic acid	1.05

Since rats in experiments on unsaturated fatty acids usually received somewhat less than 1 g. of yeast per day the B vitamin adequacy of the diets may have been borderline in some respects and, as a source of biotin, quite sub-optimal. In this regard Burr's observation on the superior efficacy of liver should be recalled (116) as well as the curative properties of a rice bran concentrate (136). Further, MacKay and Barnes (142) contributed the interesting observation that the dermal disorders manifest in rats which were fed 10% egg white could be completely cured by the replacement of some of the dietary crisco with corn oil in addition to the provision of extra pyridoxin. The diet used contained 5% yeast, a quantity which would provide suboptimal amounts of pyridoxin. The possibility of the synthesis of essential fatty acids in long-term experiments in which optimal amounts of all of the B vitamins are provided should be the subject of further investigation.

X. Summary

In recent years experimental data have shown that several of the B vitamins are necessary for the *in vivo* synthesis of fats from carbohydrates or protein. This is not surprising since thiamin, riboflavin, and nicotinic acid have been proven to be constituents of enzyme systems which are essential for metabolism. It is entirely likely that pyridoxin, pantothenic acid, and biotin act in a similar capacity. While little is definitely known regarding the chain of reactions by which fat is formed from protein it has been assumed for some years that the formation of fat from carbohydrate begins after the degradation of carbohydrate has proceeded to the pyruvic acid stage; the carboxylase system, containing thiamin, is necessary for the utilization of pyruvic acid. To regard the effects of the B vitamins upon fat synthesis as fortuitous adjuncts of "growth" produced by these vitamins is an attitude which overlooks what is already known about the fundamental enzymatic activities of the B vitamins and which might retard an advancement in knowledge of metabolism. Most of the B vitamins are now available as pure chemical substances. What is now needed is not continued emphasis upon "growth", or skin lesions, or other superficial phenomena, but research upon the fundamental biochemical reactions in which these

vitamins serve, and derangement of which are responsible for the superficial lesions. The splendid success of attempts to isolate and synthesize various vitamins has opened new pathways and provided more exact methods for the study of metabolism. The sequence of events in the intermediary metabolism of carbohydrates, proteins, and fats has long remained obscure. New opportunities are available for the study of metabolism; old methods and concepts may suffer. If many of the vitamins, acting as integral parts of enzyme systems, are necessary for metabolic reactions, these vitamins should be considered in studies on metabolism. In future investigations on fat synthesis and metabolism it will no longer be sufficient to state that the animals were fed "an adequate diet"; careful consideration should be given to the B vitamin supplements, both qualitatively and quantitatively.

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