ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/233773345

The Discovery and Characterization of Riboflavin

Article *in* Annals of Nutrition and Metabolism · November 2012

DOI: 10.1159/000343111 · Source: PubMed

CITATIONS
8

READS
1,513

2 authors:

Christine Clewes
Free lance
66 PUBLICATIONS **2,327** CITATIONS
SEE PROFILE

David Thurnham
Ulster University
222 PUBLICATIONS **6,823** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

NUSTART Project View project

BRINDA I View project

All content following this page was uploaded by Christine Clewes on 07 March 2016.

The user has requested enhancement of the downloaded file.

Annals of Nutrition & Metabolism

Ann Nutr Metab 2012;61:224–230
DOI: 10.1159/000343111

Published online: November 26, 2012

The Discovery and Characterization of Riboflavin

Christine A. Northrop-Clewes[a] David I. Thurnham[b]

[a]Nutrition Consultant, Cambridge, and [b]Northern Ireland Centre for Food and Nutrition, University of Ulster, Coleraine, UK

© *Free Author Copy – for personal use only*

ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.
Written permission to distribute the PDF will be granted against payment of a permission fee, which is based on the number of accesses required. Please contact permission@karger.ch

Key Words
Riboflavin · Lactoflavin · Vitamin B_2 · Growth promoting

Abstract
The first observation of a pigment in milk with yellow-green fluorescence can be traced to the English chemist Alexander Wynter Blyth in 1872, but it was not until the early 1930s that the substance was characterized as riboflavin. Interest in accessory food factors began in the latter half of the 19th century with the discovery of the first vitamin, thiamin. Thiamin was water soluble and given the name vitamin B_1. However, researchers realized that there were one or more additional water-soluble factors and these were called the vitamin B-2 complex. The search to identify these accessory food factors in milk, whole wheat, yeast, and liver began in the early 1900s. As there is no classical nutritional disease attributable to riboflavin deficiency, it was the growth-stimulating properties of the food extracts given to young rats that provided the tool with which to investigate and eventually extract riboflavin. Riboflavin was the second vitamin to be isolated and the first from the vitamin B-2 complex; the essential nature of the vitamin as a food constituent for man was shown in 1939.

Copyright © 2012 S. Karger AG, Basel

Introduction

Riboflavin, also known as vitamin B_2, is a water-soluble, yellow-orange organic compound in the vitamin B complex that is required for a number of metabolic activities. Plants and many microorganisms are able to synthesize riboflavin, but animals must get this essential nutrient from their diet, e.g. milk, leafy vegetables, whole grains, liver, egg white, cheese, and fresh meat. Although needed only in small amounts, riboflavin is essential to all animals and deficiency is known as ariboflavinosis. In man, deficiency is associated with cracking of the skin at the corners of the mouth and fissuring of the lips, swollen red beefy tongue, corneal vascularization and sensitivity of eyes to light, itching and scaling of the facial skin. However, there is no clear associated disease and deficiency has never been fatal.

Riboflavin is the central component of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), and like the other B vitamins, it plays a key role in energy metabolism, especially metabolism of fats, ketone bodies, carbohydrates, and proteins. Almost all of the flavin coenzyme released by enzyme turnover is reutilized. It is involved in the support of the immune

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2012 S. Karger AG, Basel
0250–6807/12/0613–0224$38.00/0

Accessible online at:
www.karger.com/anm

Dr. Christine Clewes
46 High Street, Little Wilbraham
Cambridge CB21 5JY (UK)
E-Mail christinaclewes@btinternet.com

and nervous systems, formation of red blood cells, cell reproduction, and activation of folate and pyridoxine (vitamin B_6).

Riboflavin is one of the more stable vitamins, but can be readily destroyed by ultraviolet rays or sunlight.

History

Accessory Factors in Food and Deficiency Diseases

The need for the major food groups of protein, carbohydrate, and fat to nourish our bodies and of minerals to maintain the skeleton was appreciated by the latter half of the 19th century. However, recognition of essential micronutrients only started to unfold when research started to unravel the causes of the major deficiency diseases. Christiaan Eijkman (1858–1930) demonstrated in 1897 that a paralytic condition closely resembling the polyneuritic symptoms of beriberi could be produced in chickens by feeding them both stale as well as freshly cooked polished rice [1]. Following this discovery, Casimir Funk (1884–1967), in 1911, was the first person to report the isolation of a substance in rice polishings that had antiberiberi properties. As this substance could be shown to have an amino group within its structure, he called it a 'vital amine'. His work introduced the word 'vitamine' to describe a substance present in the diet in small amounts but essential for life.

No nutritional deficiency disease like beriberi, pellagra or xerophthalmia led to the research and discovery of riboflavin. Even today, riboflavin deficiency is probably one of the commonest nutritional deficiencies in the developing world, yet little attention is paid to it as it is not linked to any serious clinical condition [2]. In man, early symptoms are usually mild disturbances of the skin and mucous membranes, but experimentally, deficiency of riboflavin in animals brings about a rapid restriction of growth. The effects on growth may equally apply in man and delay the development of serious clinical symptoms by lowering requirements. However, experimentally, it was the impairment of growth when animals were placed on semi-synthetic diets that initiated the research leading to the characterization of riboflavin.

In 1879, Alexander Wynter Blyth, an English chemist, is credited as being the first person to isolate a water-soluble material from cow milk whey that glowed with a yellow-green fluorescence when exposed to light and gave it the name lactochrome [3], 'lacto' from the milk and 'chrome' meaning color because of the yellow pigment. However, at that time Blyth was not able to determine the chemical composition or properties of lactochrome, and it was not until the 20th century that workers began to look more closely at the yellow pigment when research on accessory factors really began (table 1).

Nutritional Completeness of Individual Foods

In the early 1900s, a number of workers were investigating what was necessary to maintain growth in rats. Among them were Frederick Gowland Hopkins (1861–1947) and Elmer McCollum (1879–1967). Hopkins is reported as suggesting that purified diets lack an organic nutrient that could be supplied by a small quantity of milk but did not follow up the observation [4]. McCollum, on the other hand, describes how, in 1907, he reviewed the literature starting from 1870 and realized that animals (mostly mice) reared on purified food stuffs such as protein, fats, carbohydrate, and inorganic salts failed rapidly and died [5]. During the course of this work he too noted the protective properties of milk and realized that no one had attempted to determine the completeness of individual natural food substances as a sole source of nutrition for an animal. To this end, he started a series of experiments with rats to determine which foods contained essential elements to supplement the purified diet. Subsequently, McCollum and assistant Marguerite Davis (1887–1967) [6] produced three papers in 1915 which showed a diet containing 2% of wheat embryo or milk powder with polished rice, casein, salts, and butter fat provided enough of an 'essential accessory' to support growth of young rats [6–8]. The accessory substance was soluble in water and in alcohol and was stable to heat. The authors concluded that 'unidentified substances' were indispensable for growth and prolonged maintenance in young rats [7]. They also showed that the water-soluble accessory factor in milk whey and wheat embryo could be heated by autoclave at 15 pounds pressure for 1 h without loss of activity [8]. By 1916, McCollum and his graduate student Cornelia Kennedy thought that there were two accessory factors in the diet: water-soluble B, found in milk, egg yolk and wheat embryo, and fat-soluble A, found in foods like butter fat, egg yolk, and fish oil. At the time, McCollum thought that his water-soluble B was the same factor as the anti-beriberi substance described earlier by Christiaan Eijkman [5].

Confusion concerning Vitamin B-2 Complex and Anti-Pellagra Properties

Overlapping in time with McCollum, Joseph Goldberger (1874–1929) was put in charge of investigating the cause of pellagra in the southern states of the USA.

Table 1. Milestones in the discovery of riboflavin

Date	Researcher	Observation
1897	Blyth	Isolation of lactochrome, a water-soluble, yellow fluorescent material from milk whey
1906	Hopkins	Synthetic diets lacked an organic nutrient present in minute amounts in milk that stimulated appetite, food consumption and growth in rats and mice
1915	McCollum	Proposed that there were two accessory factors in diet: a water-soluble B and a fat-soluble A. Believed that B was the anti-beriberi vitamin B_1 identified by Eijkman
1927	Goldberger	Proposed there was an anti-pellagra factor in eggs, milk, etc., and it was the same substance as 'water-soluble B' identified by McCollum
1927	Chick and Roscoe	Proposed the water-soluble B identified by McCollum was two factors: anti-beriberi B_1 and the B-2 factor
1932	György	Suggested B-2 factor comprised an anti-pellagra factor and growth-promoting factor
1932	Warburg and Christian	Extracted a yellow enzyme from yeast and demonstrated the yellow color was dialyzable and could be recoupled to the enzyme
1933	Kuhn	Isolation of lactoflavin from milk. Lactoflavin was the growth component in B-2 factor and had the same characteristics as the factor isolated by Blyth
1934–35	Kuhn and Karrer	Independently described the structure of riboflavin
1935	Birch, György, and Harris	Differentiated the anti-pellagra factor from the growth-promoting vitamin B-2 factor (riboflavin)
1935	Theorell	Riboflavin in the yellow enzyme was in the form of FMN
1939	Sebrell and Harris	Demonstrated riboflavin was essential in man
1968	Glatzle	Proposed the use of the glutathione reductase test as a functional measurement of riboflavin status

Sufferers developed severe skin eruptions on parts of their body exposed to strong sunlight. There were mental changes, which put people into asylums, and there was a high death rate. Sufferers consumed corn (maize), and the cause was popularly believed to be an infection from insects in the corn. Goldberger noted, however, the poverty and monotony of the diets consumed by sufferers and initially believed that the disease was due to inferior dietary protein. However, prevention of human pellagra with proteins proved disappointing, but supplements of eggs and milk, meat [9] and later yeast were able to prevent and cure pellagra. Experimentally, he also showed that the substance in yeast was able to maintain health in young rats and prevent the decline and death, which was preceded by severe skin lesions. Goldberger called the substance the pellagra-preventative or P-P dietary factor, and in 1927, he thought his factor was the same as the water-soluble vitamin B_2 identified by McCollum and coworkers.

In 1927, the British Committee on Accessory Food Factors defined vitamin B_2 as 'the more heat-stable, water soluble dietary factor recently described and named pellagra-preventive (P-P) factor by Goldberger and colleagues in 1926'. The committee noted that the latter workers found the factor necessary for the maintenance and prevention of characteristic skin lesions in rats raised on semi-synthetic diets which they likened to the lesions in human pellagra [10]. However, at the same time, Harriette Chick (1875–1977) and Margaret Honora Roscoe (born 1903) [11, 12] reviewed the literature to date and suggested that the water-soluble B, identified by McCollum and Goldberger, was not one substance but at least two: (1) an anti-neuritic (anti-beriberi) vitamin B_1 and (2) vitamin B_2, another water-soluble vitamin. They pointed out that some foods were rich in anti-neuritic properties but poorly supported growth and vice versa. For example, yeast was a rich source of both B vitamin properties, but wheat embryo was rich in the anti-neuritic component but poor in the water-soluble vitamin B/P-P factor [11].

Identification of Riboflavin and Separation from Anti-Pellagra Factor

By the end of 1932, Paul György (1893–1976) reported that three separate components had been identified in the vitamin B complex as needed by the rat: (1) vitamin B_1, the anti-neuritic factor; (2) vitamin B_4 which prevented loss of coordination and ataxia, and (3) vitamin B_2, the anti-pellagra factor [13]. However, at the same time, György noted that vitamin B_2 may be further divisible into two: one factor that was predominantly growth promoting and the second, the anti-pellagra factor [12, 13]. Vitamin B_2 was noted because of its high thermostability

compared to vitamins B_1 and B_4, but all three showed growth-promoting properties under 'suitable circumstances' [13]. Chick and Alice Mary Copping (1906–1996), in 1930, had shown that an alkali- and heat-stable, water-soluble, growth-promoting factor called factor Y probably existed, but its place in the B complex was not determined [14]. It was subsequently noted by Thomas William Birch et al. [15] that vitamin B_4 deficiency was probably due to a chronically deficient thiamin state since the signs disappeared when thiamin was given in a sufficiently large dose.

The extraordinary difficulties faced by workers in this field to carry out reproducible experiments is illustrated in the paper of Chick and Roscoe [12] who were trying to deprive young rats of vitamin B_2 and obtain reproducible skin lesions. In early experiments, young rats fed a purified diet plus a source of vitamin B_1 failed to grow and, within 7 weeks, developed dermatitis. In later experiments, skin symptoms were absent or irregular. Limiting the supply of water-soluble vitamins to the dam produced no improvement in consistency of clinical outcome when the pups were used, and it was not until a rigorous purification procedure of the milk protein was introduced that a consistent inhibition of growth was achieved and skin symptoms gradually developed from the 5th week [12].

Paul György began his intensive work on the vitamin B_2 factor with two objectives: (1) to chemically isolate the growth-promoting factor, which he did in collaboration with Richard Kuhn (1900–1967) and Julius Wagner-Jauregg (1857–1940) at the University of Heidelberg, and (2) to show that the pellagra-like dermatitis was due to a different factor. The fact that many workers had difficulty in reproducing the skin lesions which Goldberger attributed to an anti-pellagra factor suggested to György that the growth restriction and dermatitis were due to different factors [13]. For all these studies, they used a synthetic diet originally devised by Anne Bourquin (born 1897) and Henry Clapp Sherman (1875–1955) in 1931, which contained thiamin but lacked the B-2 complex [16]. On that diet, the weight curves of young rats soon flattened out or showed a decline, and addition of crude extracts of yeast, rice bran, liver, milk concentrates or whey (all autoclaved to destroy thiamin) all restored normal growth. To test each extract from each of these foods for growth-promoting activity required a period of 3–4 weeks and a constant supply of prepared animals. Fortunately, an early observation established that one of the active growth-promoting components (riboflavin) in the growth experiments had a yellow-green fluorescence which was visible in near-neutral solution and was destroyed by exposure to visible light for 6–24 h [10]. Using these characteristics, they were able to identify lactoflavin, which was first prepared in crystalline form from milk.

'Active Yeast'

In the course of the above work, György and colleagues found that when the diet of Bourquin and Sherman was used and growth in rats became stationary, it was not possible to restimulate growth by addition of lactoflavin alone. György and his colleagues then used the Bourquin and Sherman diet plus 'active yeast' (which was yeast from which all lactoflavin had been removed), but it still produced no growth. However, providing the Bourquin and Sherman diet plus the 'active yeast' and lactoflavin to the growth-stationary rats did produce a normal growth response. György therefore used the Bourquin and Sherman diet plus the 'active yeast' to test their food extracts for growth promoting activity [13].

During the chemical isolation of lactoflavin, not a single rat developed the pellagra-like dermatitis but, as the experiments were too short for the appearance of clinical symptoms, it was still possible that lactoflavin had anti-pellagra properties [13]. Around this time, however, commercially prepared crystalline thiamin became available, and to overcome some of the shortcomings in the diet of Bourquin and Sherman, in which the vitamin B_1 content was largely uncontrolled, an additional amount of thiamin was added to the experimental diet. Having removed an element of uncertainty about the vitamin B_1 status of the animals, the addition of 'active yeast' was no longer necessary, and György found that the appearance of the pellagra-like dermatitis reported by Goldberger could be reproducibly obtained by a B_2-deficient diet whether or not lactoflavin was present. It appeared that the pellagra-like dermatitis was a feature of a vitamin B_2-deficient rat, but its appearance was erratic when rats had marginally adequate thiamin status (the Bourquin and Sherman diet) and independent of the growth-promoting properties of lactoflavin [13].

A lack of riboflavin in young rats manifests itself first by retardation of growth and later complete cessation of growth. Effects on the skin are not very striking or specific. They do have a definite seborrheic quality but are certainly not characteristic of a pellagra-like disease [10]. However, working with Birch and Leslie Julius Harris (born 1898), György and colleagues were able to show independent of and simultaneously with Conrad Elvehjem's (1901–1962) group in Wisconsin that riboflavin was different from the specific pellagra-preventing factor of

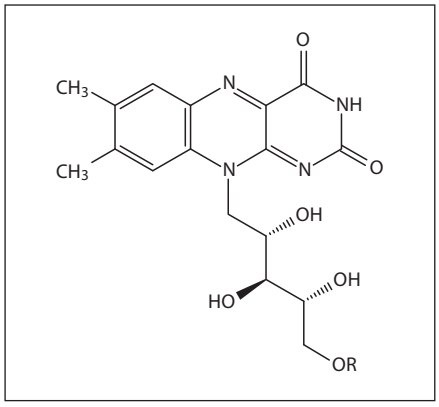

Fig. 1. Riboflavin and its coenzymes FMN and FAD. The compound shown is an isoalloxazine molecule linked to a molecule of ribose in its reduced form (ribityl) through the nitrogen atom at position number 10. 'R' represents hydrogen, phosphate or adenosine diphosphate linked through its phosphate group; the respective compounds are riboflavin, FMN, and FAD.

Goldberger and colleagues. Elvehjem's group went on to identify nicotinic acid (niacin) as the pellagra-preventing component in the vitamin B-2 complex [15, 17].

Riboflavin and Role in Tissues

In papers written by György, Kuhn, and Wagner-Jauregg (1933, 1934) [18] two new pieces of information were reported: (1) vitamin B_2 was not a single substance, and (2) it was possible to identify one of the accessory food factors in crystalline form prepared from milk. The first isolation of the colored component was from milk in 1933 [19], and it was termed lactoflavin and corresponded to the lactochrome of Blyth. Similar crystalline compounds termed ovaflavin (from egg) [20] and hepatoflavin (from liver) soon followed. Kurt Günter Stern (born 1904) was first to suggest that the structure of the substance was a derivative of isoalloxazine [21, 22], and this was confirmed independently by Kuhn [23, 24] and Paul Karrer (1889–1971) [25] when it was realized that all three compounds were chemically identical. The biologically active 'flavin' was found to be a derivative of isoalloxazine with two methyl groups and a sugar (pentose) radical attached. As the sugar was ribose, the name riboflavin was proposed and adopted in 1937 by the Council of Pharmacy and Chemistry of the American Medical Association (fig. 1) [10]. However, riboflavin still retains the name vitamin B_2 as it was the first vitamin isolated from the B-2 complex.

The isolation and identification of lactoflavin at the University of Heidelberg under the guidance of Kuhn and György makes interesting reading. György initially asked Kuhn to isolate vitamin B_2 from the livers of rats, but they quickly expanded their efforts, purifying flavin pigments from plants and animal materials. Extraction with traditional solvents proved ineffective, and co-worker Edgar Lederer (1908–1988) began to develop new chromatographic techniques to purify the materials. Even with the new methods, purification was an immense undertaking. Although flavins are widespread pigments in nature, they are only present in extremely small concentrations. In fact, the isolation and purification of 1 g of the 'beautiful yellow substance', as Kuhn called it, required >5,000 liters of milk or the dried albumin from 34,000 eggs [18]. Lederer was forced to flee to France in March of 1933, and Wagner-Jauregg took over the collaboration with György. He isolated flavins in yeast, heart muscle, and a variety of plant materials. The physics director, Karl Wilhelm Hausser (1887–1933), also in Heidelberg at the Kaiser Wilhelm Institute for Medical Research, worked closely with the Kuhn group on the spectral analysis of the purified substances, and Friedrich Weygand (1911–1969), Hermann Rudy, and later Rudolf Ströbele and Pierre Desnuelle (1911–1986) made significant contributions as Kuhn expanded the scope of the investigation to include clarification of the chemistry and functions of these molecules [18].

From the beginning, Kuhn assumed a fixed relationship between the growth-promoting components of vitamin B_2 and the flavins, especially after they were able to correlate the absorption patterns of the flavin pigments to the absorption characteristics of vitamin B_2. Determining if the flavins were precursors, or the vitamin itself was difficult because crystallizing the lactoflavin, hindered the structural analysis. However, the team discovered a breakdown product of lactoflavin, lumiflavin, in which the ribose residue was removed by irradiation with ultraviolet light, which they were able to crystallize. After determining the structure of lumiflavin, they were able to extrapolate enough information to synthesize lactoflavin and determine its composition. Finally, they had proof that lactoflavin was riboflavin, not a precursor of the growth factor in vitamin B_2. Indeed, in experimental tests with rats, Kuhn's crystallized flavins proved to be the most active preparation of vitamin B_2 yet discovered [18].

Riboflavin Coenzymes

The question remained as to how riboflavin stimulated growth and other bioactivity. The key to finding the answer was once again the breakdown product lumiflavin. In 1932, Otto Heinrich Warburg (1883–1970) and Walter Christian extracted a yellow enzyme ('Gelbes

Ferment') from brewer's yeast during attempts to elucidate the nature of the biological oxidations and reported that the 'Gelbe Ferment' enabled them to link the oxidation of glucose-6-phosphate (G6P) to molecular oxygen [26]. They found that when G6P was oxidized by methylene blue in the presence of two erythrocyte components, an enzyme ('Zwischenferment', G6P dehydrogenase), and a small heat-stable 'Coferment' ($NADP^+$), adding the 'Gelbes Ferment' permitted the system to form a complete respiratory chain reacting with molecular oxygen (fig. 2). Warburg had discovered an important oxidation enzyme and also found that if the enzyme was dialyzed, the yellow color and enzyme activity were lost. Kuhn, who had remained in close contact with Warburg, quickly realized that his lumiflavin was probably the yellow component of Warburg's oxidation enzyme and proposed that vitamin B_2 was its precursor. Follow-up tests showed that rats given lumiflavin and Warburg's enzyme produced similar increased growth rates. The research of Kuhn and Rudy from 1934 to 1936 helped to show that lumiflavin played an enzymatic role in the hydrogenation of lactic acid, pyruvic acid, and succinic acid (major contributions were also made by Warburg and Christian). These are all important reactions involved in cell respiration. In addition, by combining a FMN with a protein moiety of Warburg's yellow enzyme, Kuhn and Rudy produced the very first partial synthesis of a fully functional enzyme. This was the first suggestion of a reversible relationship between vitamins and enzymes [18, 27].

The 'old yellow enzyme' was purified by Hugo Theorell (1903–1982) in 1935 using mostly column chromatography and was reported to be a colorless apoprotein and yellow dye, both essential for enzyme activity. This finding helped to establish the essential role of proteins in enzyme catalysis and the 'old yellow enzyme' provided the first biochemical role for a vitamin. In his Nobel Prize Lecture in 1955, Theorell [28] recounted that the yellow cofactor was in fact riboflavin 5′-phosphate, now also termed FMN (fig. 1). Since the 1950s, the 'old yellow enzyme' has been characterized by Vincent Massey (born 1926) and his co-workers, and a considerable amount has been discovered about its metabolism. In 1969, Rowena G. Matthews and Massey described a new method for the isolation of electrophoretically homogeneous 'old yellow enzyme' ($NADPH_2$ oxidoreductase, EC 1.6.99.1) from brewer's bottom yeast [29]. 'Old yellow enzyme' was bound in a charge transfer complex to an unidentified compound of low molecular weight, which could be dissociated by dialysis of the reduced enzyme, and reformed by the addition of the dialysate to the oxidized enzyme.

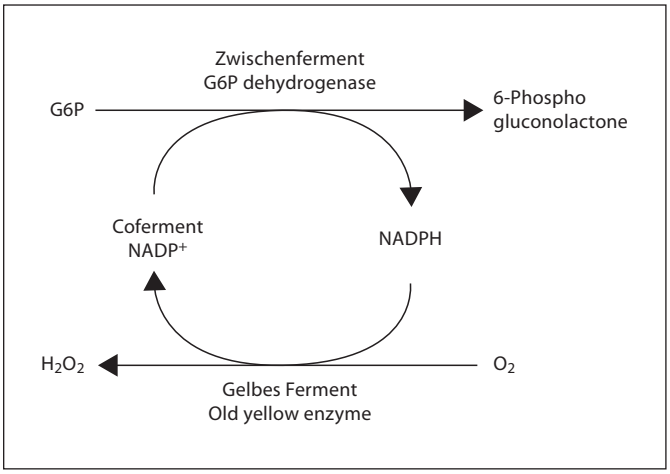

Fig. 2. Reaction system described by Warburg and Christian [26] in 1932. The reaction shows that when G6P is oxidized by methylene blue in the presence of G6P dehydrogenase and NADP from erythrocytes, addition of the 'Gelbe Ferment' from yeast enabled the system to complete a respiratory chain by reacting with molecular oxygen.

Riboflavin was the first member of the vitamin B-2 complex that was isolated and identified. It is not surprising that it continues to be called vitamin B_2 without any reference to the comprehensive character of the original term [10]. The essential nature of the riboflavin as an exogenous food constituent for the human organism was eventually proved by the studies of William Henry Sebrell (1901–1992) and Roy Edwin Butler (1902–1974) in 1939 [30]. These workers reported that 13 out of 18 women who received a diet low in riboflavin developed a reddened, denuded lesion of the lips (cheilosis), maceration and fissuring of the angles of the mouth (angular stomatitis), and seborrheic accumulations at the nasolabial folds. These lesions disappeared following daily administration of synthetic riboflavin, reappeared on discontinuation of the riboflavin, and disappeared again on reinstitution of the riboflavin treatment.

The red-cell glutathione reductase enzyme stimulation test described by Glatzle [31, 32] has proved a reliable test of riboflavin status and a better measure of riboflavin deficiency than the presence of clinical lesions. Nevertheless, the presence of clinical signs of riboflavin deficiency backed up by the enzyme stimulation test is still a useful procedure for assessing the riboflavin status in a community [33], and a modification of the method is currently being used in the UK National Diet and Nutrition Survey to assess the population riboflavin status.

References

1 Thurnham DI: Beriberi; in Caballero B, Allen L, Prentice A (eds): Encyclopedia of Human Nutrition, ed 2. Oxford, Elsevier, 2005, pp 269–278.
2 Bates CJ: Human riboflavin requirements, and metabolic consequences of deficiency in man and animals. World Rev Nutr Diet 1987; 50:215–265.
3 DSM Nutritional Products: Vitamin basics; vitamin B_2 history. http://www.vitamin-basics.com/index.php?id=55 (accessed September 10, 2012).
4 Hopkins FG: Feeding experiments illustrating the importance of accessory factors in normal dietaries. J Physiol 1912;44:425–460.
5 McCollum EV: The paths to the discovery of vitamins A and D. J Nutr 1967;91:11–16.
6 McCollum EV, Davis M: The nature of the dietary deficiencies of rice. J Biol Chem 1915; 23:181–232.
7 McCollum EV, Davis M: The essential factors in the diet during growth. J Biol Chem 1915;23:231–256.
8 McCollum EV, Davis M: The cause of the loss of nutritive efficiency of heated milk. J Biol Chem 1915;23:247–254.
9 Goldberger J, Tanner WF: A study of the treatment and prevention of pellagra. Experiments showing the value of fresh meat and milk, the therapeutic failure of gelatin and the preventive failure of butter and of cod liver oil. US Public Health Reports 1924;39: 87–106.
10 Gyorgy P: Early experiences with riboflavin; a retrospect. Nutr Rev 1954;12:97–100.
11 Chick H, Roscoe MH: On the composite nature of the water-soluble B vitamin. Biochem J 1927;21:698–711.
12 Chick H, Roscoe MH: The dual nature of water-soluble vitamin B. II. The effect upon young rats of vitamin B_2 deficiency and a method for the biological assay of vitamin B_2. Biochem J 1928;22:790–799.
13 Gyorgy P: Investigations on the vitamin B_2 complex. I. The differentiation of lactoflavin and the 'rat antipellagra' factor. Biochem J 1935;29:741–759.
14 Chick H, Copping AM: The alcohol-solubility of the anti-dermatitis, more heat-stable vitamin B2 constituent of the vitamin B complex. Biochem J 1930;24:1744.
15 Birch TW, Gyorgy P, Harris LJ: The vitamin B_2 complex. Differentiation of the antiblacktongue and the 'P.-P.' factors from lactoflavin and vitamin B_6 (so-called 'rat pellagra' factor). Biochem J 1935;29:2930–2850.
16 Bourquin A, Sherman HC: Quantitative determination of vitamin G (B2). J Am Chem Soc 1931;53:3501.
17 Elvehjem CA, Koehn CJJ: Studies on vitamin B_2 (G). The non-identity of vitamin B_2 and flavins. J Biol Chem 1935;108:709–728.
18 Nobelprize.org: Richard Kuhn and the chemical institute: double bonds and biological mechanisms. http://www.nobelprize.org/nobel_prizes/medicine/articles/states/richard-kuhn.html (accessed September 10, 2012).
19 Kuhn R, Gyorgy P, Wagner-Jauregg T: Über eine neue Klasse von Naturfarbstoffen (Vorläufige Mitteilung). Ber Deutsch Chem Ges 1933;66:317–312.
20 Kuhn R, Gyorgy P, Wagstaff L: Über Ovoflavin, den Farbstoff des Eiklars. Ber Deutsch Chem Ges 1933;66:576–580.
21 Stern KG, Holiday ER: Die Photo-flavine, eine Gruppe von Alloxazin-Derivaten. Ber Deutsch Chem Ges 1934;67:1442–1452.
22 Stern KG, Holiday ER: Zur Konstitution des Photo-flavins; Versuche in der Alloxazin-Reihe (Vorläufige Mitteilung). Ber Deutsch Chem Ges 1934;67:1104–1106.
23 Kuhn R, Reinemund K: Über die Synthese des 6.7.9-Trimethyl-flavins (Lumi-lactoflavins). Ber Deutsch Chem Ges 1934;67:1932–1936.
24 Gyorgy P, Kuhn R, Wagner-Jauregg T: Das Vitamin B_2. Naturwissenschaften 1933;21: 560–561.
25 Karrer P, Schöpp K, Benz F, Pfaehler K: Synthesen von Flavinen III. Helv Chim Acta 1935;18:69–79.
26 Warburg O, Christian W: Ein zweites sauerstoffübertragendes Ferment und sein Absorptionsspektrum. Naturwissenschaften 1932;20:688.
27 Kuhn R, Rudy H: Katalytische Wirkung der Lactoflavin-5′-phosphorsäure; Synthese des gelben Ferments. Ber Deutsch Chem Ges 1936;69:1974–1977.
28 Theorell H: Nobel Prize lecture. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1955/theorell-lecture.pdf (accessed September 10, 2012).
29 Matthews RG, Massey V: Isolation of old yellow enzyme in free and complexed forms. J Biol Chem 1969;244:1779–1786.
30 Sebrell WH, Butler RE: Riboflavin deficiency in man (ariboflavinosis). US Public Health Reports 1939;54:2121–2131.
31 Glatzle D, Weber F, Wiss O: Enzymatic test for the detection of a riboflavin deficiency. NADPH-dependent glutathione reductase of red blood cells and its activation by FAD in vitro. Experientia 1968;24:1122.
32 Glatzle D, Körner WF, Christeller S, Wiss O: Method for the detection of biochemical riboflavin deficiency. Stimulation of NADPH2-dependent glutathione reductase from human erythrocytes by FAD in vitro. Int Z Vitaminforsch 1970;40:166–183.
33 Thurnham DI, Migasena P, Vudhivai N, Supawan V: A longitudinal study on dietary and social influences on riboflavin status in pre-school children in northeast Thailand. SE Asian J Trop Med Publ Hlth 1971;2:552–563.

© Free Author Copy – for personal use only

ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.
Written permission to distribute the PDF will be granted against payment of a permission fee, which is based on the number of accesses required. Please contact permission@karger.ch

View publication stats