

LXXXIX. FLAVIN AND THE PELLAGRA- PREVENTING FACTOR AS SEPARATE CONSTITUENTS OF A COMPLEX VITAMIN B₂.

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(Received January 31st, 1935.)

THE observations to be recorded in this paper arose in quite an indirect manner from enquiries in progress in this Laboratory on certain interrelations between vitamin function and redox systems on one side and cell activity on the other. (This work has since been continued in conjunction with Dr S. N. Ray and Dr P. György; and one aspect of it is dealt with in an accompanying paper [Ray *et al.*, 1935].) In the course of these investigations it was observed incidentally that rats fed on certain diets deficient in "vitamin B₂", modelled on the cataract-producing diet of Langston and Day [1933], developed symptoms of severe and characteristic pellagra in a large proportion of cases. As a necessary preliminary to the further prosecution of this work, and also in order to help to clarify the present position with regard to flavin and vitamin B₂, it was thought desirable to examine the nature of the several factors concerned in restoring growth and preventing pellagra on these dietary regimens. As will be shown, the results obtained have confirmed the conclusion of György [1934; 1935] that flavin is not the "rat antipellagra factor". It possesses vitamin B₂ activity in the sense that it is partly responsible for the growth-promotion observed when the usual natural sources of "heat-stable, growth-promoting vitamin B₂" are added to the rat's diet as the supplement to vitamin B₁.

Past work. The growth-promoting action of flavin was demonstrated by Kuhn *et al.* [1933]. They left it undecided whether or not flavin was identical with the antipellagra factor. György [1934] later reported that flavin possesses no antipellagra action for rats. Elvehjem and Koehn [1934] have recently reached the same conclusion using chickens. A number of earlier workers [Sure *et al.*, 1931; Thatcher *et al.*, 1931; Gurin *et al.*, 1931; Sherman and Sandels, 1929; 1931] had in fact suspected that vitamin B₂ (or G) as usually measured might be a complex; but Roscoe [1933] could find no convincing grounds for differentiating between the dermatitis-preventing principle and the factor responsible for growth in various sources of vitamin B₂.

In recent papers Kuhn and his coworkers have used the term vitamin B₂ as though synonymous with flavin; whereas Elvehjem and Koehn [1934] now propose to restrict it to the antipellagra factor. György [1934] on the other hand has proposed to name the antipellagra factor vitamin B₃ and retain vitamin B₂ for flavin.

EXPERIMENTAL.

In the first experiment to be mentioned, begun early in 1934, a group of 20 rats was put on the following diet, similar to that described by Langston and Day.

| | |
|-------------------------------------|----|
| Extracted caseinogen (Glaxo) | 18 |
| Salt mixture | 4 |
| Cod-liver oil | 2 |
| Butter-fat | 8 |
| Maize starch | 68 |

International vitamin B₁ standard, 2 "doses" per rat daily.

After three and a half months no signs of cataract were visible; but six animals had developed severe symptoms of "pellagra".¹ This took the form of a symmetrically disposed intense dermatitis accompanied by a striking scaliness, more especially noticeable on the paws, the nose and the ears. A sticky secretion accumulated in the eyes, the lids remaining half-closed. All animals had ceased to gain weight after the first 4 weeks (except two rats which became spontaneously refected, with characteristic faeces—nos. 7 and 10, Fig. 3—and grew normally and remained free from symptoms without any addition of flavin or antipellagra concentrate, or other source of vitamin B₂ to the diet).

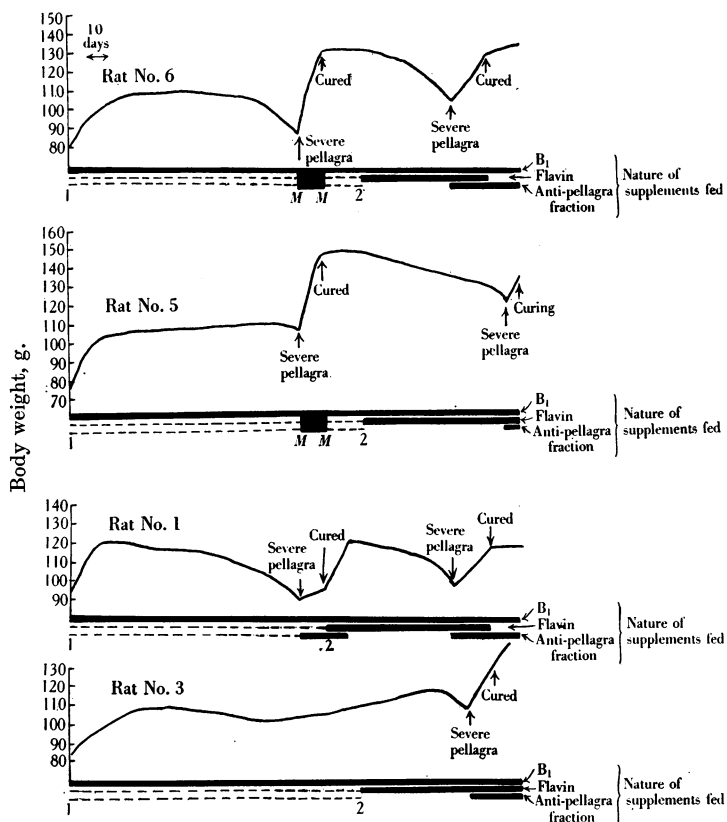


Fig. 1. Effect of different constituents of the vitamin B complex on cure or prevention of "rat pellagra", and corresponding weight curves. From 1 to 2 vitamin B₁ was given in the impure form of the International Standard, contaminated with traces of the other B-factors. After 2 it was given in a highly purified condition. At MM the entire complex was given in the form of marmite.

To test the nature of the deficiencies in this diet and their individual responsibilities for the different symptoms observed, various supplements (concentrates or natural food materials) were fed. Thus, in the first place, two animals were given the complete vitamin B complex in the form of 1.0 ml. daily of a 50 % solution of marmite (laboratory quality). The result was that the pella-

¹ Throughout this paper the term "pellagra" is used to describe this condition in rats without prejudice to the question of its identity or otherwise with pellagra in man.

grous lesions disappeared in 8–10 days in one animal, and in 12–14 days in the other and growth was resumed (see rats nos. 5 and 6, Fig. 1). The corresponding untreated controls remained uncured.

In all later work the activated acid clay (International Standard) was replaced by a purer preparation of vitamin B₁ prepared according to the method of Windaus.¹ This was found to permit the more regular production of symptoms. It may be concluded from this finding that the International Standard contains sufficient traces of the antipellagra factor to delay the regular appearance of symptoms. It may also possibly contain other "B"-factors as impurities.

Apart from purified vitamin B₁, the other active fractions employed were (1) a crystalline preparation of lactoflavin, and (2) the further "supplementary fraction" used by Kuhn *et al.* ("Peters's eluate"). The latter contains the antipellagra principle: it is free from flavin, but contains additional vitamin B₁, and possibly also other "B"-factors.

Results.

To save space, the principal results are summarised in the form of charts (Figs. 1–3) and in Table I.

Table I. *Results of prophylactic and curative tests.*

| Nature of supplements given in prophylactic tests | Result | Further additions made in curative tests | Result |
|--|--|--|---|
| Impure vitamin B ₁ (as Donath and Jansen's acid clay absorbate) Vitamin B ₁ | Loss in weight; pellagra in many cases | (1) Flavin | Some improvement in weight but no cure of pellagra Pellagra cured but weight curves still subnormal Pellagra cured and weight curves normal |
| | | (2) "Peters's eluate" | |
| | | (3) Flavin + "Peters's eluate" | |
| | | (4) B complex as marmite | |
| Vitamin B ₁ + flavin | Pellagra in all cases | "Peters's eluate" | Pellagra cured and weight curves normal |
| Vitamin B ₁ + "Peters's eluate" | No pellagra, but weight curves subnormal | Flavin | |
| Vitamin B ₁ + flavin + "Peters's eluate" | No pellagra; weight curves normal | — | |

DISCUSSION.

The above results confirm the finding of György that flavin does not prevent or cure pellagra in rats. The pellagra-preventing factor is present in the "Peters eluate". For normal nutrition, both flavin and the antipellagra factor must be present simultaneously. Uncomplicated deficiency of flavin (*i.e.* in the presence of vitamin B₁ plus the antipellagra supplement) was mainly manifested, in our experiments, by the loss of weight; and the rather unspecific skin lesions reported by György were not a prominent feature.

Vitamin B₂ nomenclature.

According to the definition given by the Committee on Accessory Food Factors [1927] vitamin B₂ is "the more heat-stable, water-soluble dietary factor,

¹ We are indebted to the I.G. Fabenindustrie of Germany for the supplies of purified vitamin B₁, crystalline flavin and "Peters's eluate".

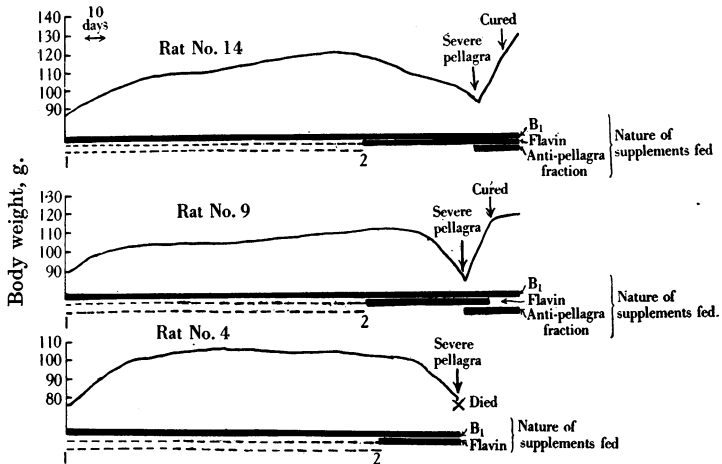


Fig. 2. The dietary supplements are indicated as in Fig. 1.

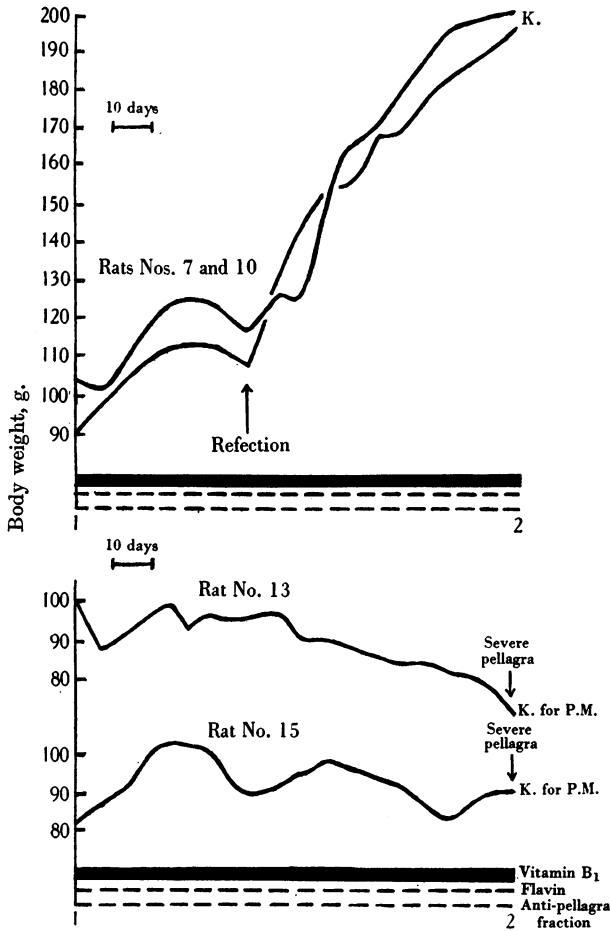


Fig. 3. Above, synthesis of vitamin B₂ complex by refection, with prevention of pellagra
Below, unrefected controls.

recently described and named P-P ('pellagra-preventive') factor by Goldberger, Wheeler, Lillie and Rogers (1926) and found necessary for maintenance of growth and health and prevention of characteristic skin lesions in rats, and considered by the latter workers to be concerned in the prevention of human pellagra". Now, in a large proportion of past experimental work the criterion of vitamin B₂ activity has not been antidermatitis action (since the symptoms of dermatitis were found difficult to produce in experimental animals), but has been rather the restoration of growth. More concretely, vitamin B₂ activity has been measured in actual practice either by the growth-promoting action or by the antidermatitis action of a material such as autoclaved yeast fed as a "heat-stable supplement" to vitamin B₁. In this sense therefore both flavin and the antipellagra factor are constituents of vitamin B₂. Normal growth is impossible in the absence of either flavin or the antipellagra factor.

It has been suggested that the term vitamin B₂ should now be restricted to the antipellagra factor [Elvehjem and Koehn, 1934]. A serious objection to this suggestion is that it would render the bulk of past work (in which growth tests have been the criterion) confusing, if not unintelligible, to future readers. On the other hand it has alternatively been recommended that the term vitamin B₂ should be applied to flavin, and indeed this has already become the current practice in many German papers. This also seems unfortunate, since vitamin B₂ has come to acquire so definite an association with the prevention of pellagra, or pellagra-like lesions. The only logical solution, which will avoid confusion, and render the past literature intelligible, is to retain the term vitamin B₂ for this complex, and to have separate names for the two (or possibly more) constituents. As flavin ("lactoflavin" of Kuhn) already has a chemical name it could be referred to simply as such; or for convenience the two constituents might perhaps be referred to as "vitamins B₂ (F)" and "B₂ (P)", or more shortly vitamins BF and BP; "F" recalling "flavin", and "P" "pellagra" (or rather the "pellagra-like lesions" in experimental animals). (The term "P-P" is already well known and may be kept for the pellagra-preventive factor in man, which further work will probably prove to be identical with the "BP"-factor which prevents the "pellagra-like lesions" in experimental animals.) Thus vitamin BF replaces the vitamin B₂ of Kuhn *et al.*; and vitamin BP replaces the vitamin B₂ of Elvehjem (=the vitamin "B₆" of György; probably identical also with "factor Y" of Chick).

vitamin B₂ $\left\{ \begin{array}{l} \text{vit. BF (=flavin= vit. B}_2 \text{ of Kuhn } et al.). \\ \text{vit. BP (= "pellagra factor" = vit. B}_2 \text{ of Elvehjem = vit. B}_6 \text{ (=?) Y (=?) P-P).} \\ \text{—} \end{array} \right.$

Final decisions on nomenclature should rest with an international body, such as the Committee on Vitamin Standardisation of the Health Department of the League of Nations. The exact letters chosen do not matter greatly. But in order to preserve continuity in the literature it should be recognised that past work on vitamin B₂ has in fact related to a complex and that new names should therefore be given to the several constituents.

SUMMARY AND CONCLUSIONS.

During the course of experiments on the interrelations between "vitamin B₂" and the redox systems in the eye, rats were fed on a vitamin B₂-deficient diet similar to that used by Langston and Day for the production of cataract, but with the vitamin B₁ provided in the form of the International Standard. It was observed incidentally that about 30 % of these rats developed symptoms of

severe and characteristic pellagra after 3 to 4 months. The opportunity was therefore taken to investigate the antipellagra properties and growth-promoting actions of the different fractions of the vitamin B complex. These were added separately and in different possible combinations, both in curative and in preventive tests, so that their individual functions might be analysed. (In later experiments purer preparations of vitamin B₁ were used and found to lead to the more regular production of symptoms—a result indicating that the International Standard is contaminated with the antipellagra vitamin and possibly with other “B”-factors.) The following conclusions were reached.

(1) *Flavin*. It is confirmed that flavin has no antipellagra action. It is however a constituent of the vitamin B₂ complex in the sense that it causes some improvement in the weight curve when given as a supplement to vitamin B₁. For adequate growth however it was necessary to supplement the vitamin B₁ plus flavin with a third fraction, which possesses antipellagra properties. Following Kuhn *et al.* [1933] this was given in the form of “Peters’s eluate”. With vitamin B₁ and flavin alone all rats without exception developed severe pellagra (eight cases).

(2) *Pellagra-preventing factor*. With vitamin B₁ plus the pellagra-preventing fraction only (“Peters’s eluate”) pellagra was always prevented or cured (thirteen cases). Without the further addition of flavin, however, body weights remained subnormal.

(3) The combined effect of vitamin B₁ plus flavin plus “Peters’s eluate” (containing the antipellagra factor) was approximately equal, in its weight-promoting action and in the cure or prevention of symptoms, to that of the total complex given in the form of marmite.

These results confirm the finding of György that flavin does not possess antipellagra activity. Both flavin and the antipellagra factor should be regarded as separate constituents of the “heat-stable, growth-promoting vitamin B₂ complex”.

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