"We are fully aware that, if material progress is to be made in augmenting in this way the food supply of rural areas, it will be essential for the district boards, and the rural community generally, to play their part in the stocking of local waters and in their conservancy. It will be for the public health officers and for all organisations interested in the welfare of the people to disseminate a knowledge of the value of the addition of fish to diet. But without some expert authority at provincial headquarters, there will be a risk that ill-advised experiments in stocking may be made and the resultant failures will seriously endanger the prospect of success for the movement as a whole.

"Improvement in the cultivator's diet holds out such promise of improvement in his general health and the addition of fish to his diet impresses us as being so much the most promising way of providing it over large areas of the country, that we consider that we are more than justified in making recommendations which, to those who know the difficulties, may well appear to err somewhat on the side of optimism."

In our opinion the time has come when the Central Government, Governments of the various autonomous provinces, local bodies and the public at large can no longer ignore the development of Indian fisheries, and if there is *no* enthusiasm for such an enterprise in this country we should not stand in the way of the Japanese who would help the masses of India by exploiting the fisheries resources of the Bay.

The Vitamin B_2 Complex and Allied Factors.

I. Mammalian Factors.

By J. R. O'Brien and R. A. Peters.
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THOUGH many suspected that vitamin B was multiple in nature, convincing proof that this was so was not produced until 1926 when, mainly by the method of feeding supplementary foodstuffs, several workers established that at least two factors were involved in rat nutrition. Of recent times this fact has induced an extensive investigation of the water-soluble factors required not only by the rat but also by the pigeon, chick, etc. It has led to the accumulation of considerable evidence for the existence of several factors generally classified under the heading of vitamin B of which an individual animal may require at least two. Table I is a list of the different factors of the vitamin B group for which evidence has been offered:

TABLE I.

Vitamin B factors (other than vitamin B_1) so far shown to be essential for mammalian nutrition.

Rat ..	vitamin B_2 ..	{ flavin vitamin B_6–antidermatitic
	vitamin B_4 ..	(position uncertain)
Dog ..	Black tongue factor	
Man ..	Anti-pellagra factor vitamin B_6	(P-P factor of Goldberger)

At present it is important to differentiate the several factors of the rat, pigeon, chick, dog and man because a superficial similarity in chemical and physiological properties suggests but does not prove a relationship among them. Of one factor only, namely flavin, is it possible to speak with some certainty. This has been isolated in crystalline form from natural sources, particularly vitamin B_2 extracts, and its structure established by synthesis. Its physiological properties have been studied in greatest detail in the rat.

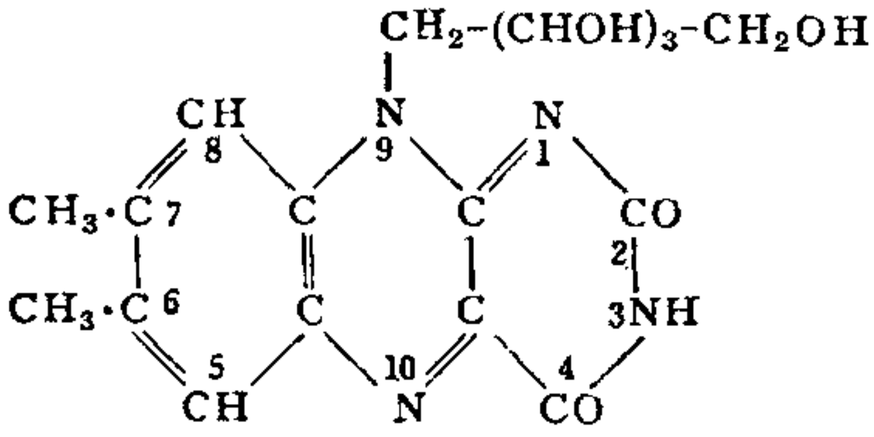
Lactoflavin.[1]

(Ovoflavin from eggs,[2,3] hepatoflavin[4] from liver, and renoflavin[5] from kidney.)

For over 80 years we have been aware of the presence of substances in animal tissues fluorescing in ultraviolet light. Many tissues contain substances fluorescing blue like quinine; Bence-Jones (1866)[6] called this property quinoidine. A preliminary investigation by Kinnersley, Peters and Squires (1925)[7] indicated that the blue fluorescence of tissues was due to more than one quinochrome (*i.e.*, substances fluorescing blue) and that those in yeast accompanied but were not identical with vitamin B_1. In 1933 a new class of natural pigments with a yellow-green fluorescence came into prominence. The biological significance

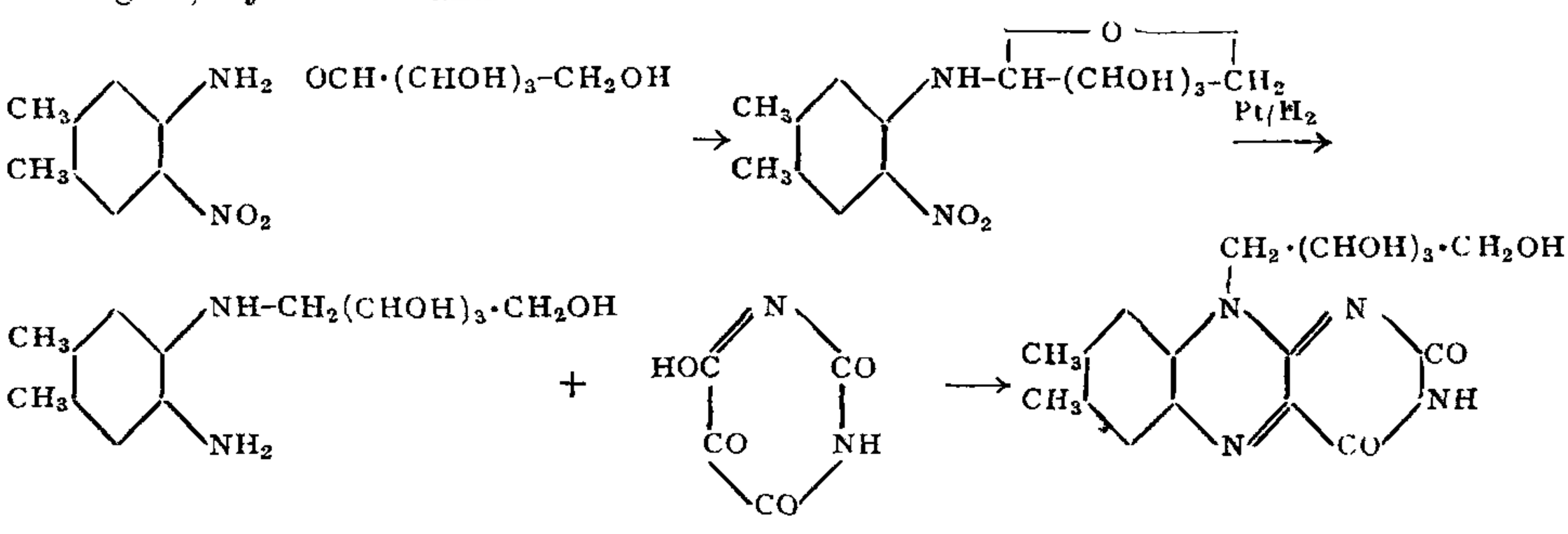
of these compounds, called flavins, was realised from the earlier isolation from yeast of an iron-free enzyme consisting of a yellow-green fluorescent component united with a protein. [Warburg and Christian (1932)[8].] Separation of the fluorescent prosthetic group, easily effected by hydrolysis, yielded an orange crystalline substance of composition $C_{17}H_{20}N_4O_6$. Meanwhile a detailed examination of the yellow-green fluorescing substances in tissues by Ellinger and Koschara[9] and Kuhn and his coworkers[10] showed that Warburg's substance was a member of a class of compounds, the lyochromes. Further emphasis on the importance of these substances was given by the report of Kuhn, György and Wagner-Jauregg[10] who, working on the nature of vitamin B_2, isolated a crystalline substance which proved to be identical in composition with the flavin of Warburg and promoted the growth of rats adequately provided with other components of the vitamin B complex. The discovery stimulated investigation into the chemistry of these compounds—some hundred or more papers being published quickly by several laboratories culminating in the synthesis of the natural product and a few of its homologues, by Karrer and Kuhn.

The steps leading to the elucidation of the structure of flavin were briefly as follows:—Warburg and Christian (1932)[8] had already shown that irradiation of flavin in alkaline solution caused its destruction with the loss of four carbon atoms and the production of lumiflavin. Upon alkaline hydrolysis lumiflavin decomposed yielding a molecule of urea. Detailed examination of the degradation products by Kuhn, Rudy and Wagner-Jauregg[11] showed that the loss of 4 carbon atoms upon irradiation arose from the breakdown of a pentose chain, confirmed the loss of urea from lumiflavin by hydrolysis and found that the acid formed simultaneously had a molecular composition suggesting a quinoxaline structure. It was therefore presumed that flavin was a dimethyl isoalloxazine containing a pentose group in the 9 position. This view was supported by evidence from a spectrographic examination of a series of alloxazine derivatives by Stern and Holiday.[12] The synthesis of flavin rapidly followed. Several homologues of the natural substance were prepared before lactoflavin itself was actually obtained. From a comparison of the biological and chemical properties of the synthetic compounds and of natural flavin the structure assigned to flavin was 6·7-dimethyl-(*d*-1′-ribityl)-*isoalloxazine*.



Flavin

The type of synthesis may be illustrated by the following series of reaction:[13]

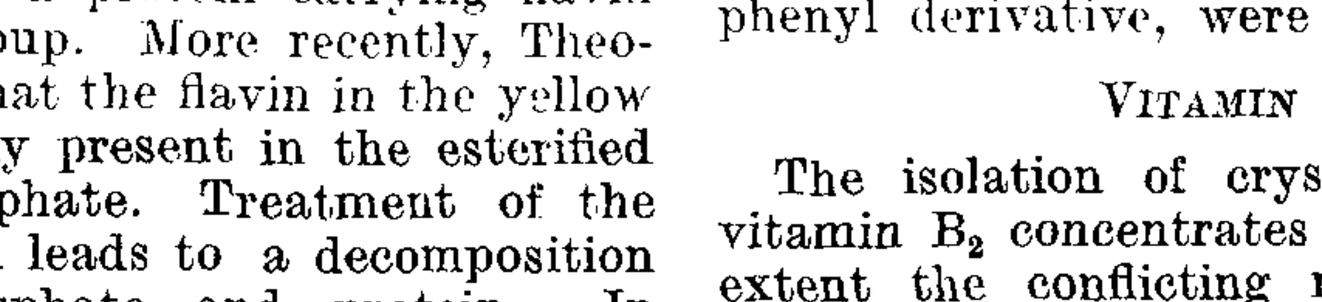


Other methods of synthesis are: Condensation of pentose with the N-mono-acyl or preferably carbethoxy-amino derivatives of dimethyl phenylene diamines.[14] After reduction with nickel and hydrogen in an autoclave, the condensation product is allowed to combine with alloxan when flavin is formed. The synthesis is achieved by Kuhn and Weygand[15] by condensing amino pentose with o-chlor nitro xylene. The labile N substituted diamine is reduced in the presence of alloxan by stannous chloride. After removal of the excess of

reducing agent the leucoflavin is oxidised by shaking with air.

The flavins are orange-yellow crystalline solids soluble in water, slightly soluble in alcohol but otherwise insoluble in organic solvents. Their characteristic feature is a yellow-green fluorescence accompanied by marked sensitivity to light.[8,10,16,17-22] According to the experimental conditions two main products arise from the irradiation of flavin solutions: (*a*) in alkaline medium, lumiflavin (6·7-dimethyl-9-methyl-isoalloxazine) and (*b*) in neutral solution, lumichrome (6·7-dimethyl-alloxazine), an intensely blue fluorescent compound.[19] Another interesting property is the reversible oxidation reduction of flavin. On treatment with hydrosulphite flavin is reduced to a colourless leuco form which is reconverted to the original yellow-green form on shaking with air. The smoothness and the ease with which these reactions proceed suggests a relation with the physiological function of flavin.

A further clue to the physiological rôle of flavin is given by the mode of combination in which it exists in various animal plant tissues. In yeast and in such organs as liver, heart, kidney, flavin exists in two forms: free flavin and flavin in a non-dialysable form. Warburg has shown that his yellow enzyme is a protein carrying flavin as a prosthetic group. More recently, Theorell[23] has found that the flavin in the yellow enzyme is actually present in the esterified form of a phosphate. Treatment of the enzyme with acid leads to a decomposition into flavin phosphate and protein. In neutral solution both these fragments recombine to give a product possessing the same activity as the original enzyme. The action of the yellow ferment has been studied by Warburg particularly with hexose monophosphate (Robison ester) as substrate. In the oxidation of hexose monophosphate to phospho-hexonic acid, flavin apparently functions as a vehicle for oxygen transportation:



A deficiency of flavin in the diet manifests itself most definitely in the rat although evidence has been presented that this substance is required by other mammals. In the rat it is now generally agreed that flavin deficiency results in a loss of appetite and cessation of growth accompanied after some weeks by the appearance of scurf-like symptoms in the vicinity of the eyes and mouth which are different from the dermatitis usually associated with lack of vitamin B_2. The hair is shed with the development of bald patches over the head and face but no swelling or inflammation of the paws occurs (Copping, 1936).[24] The daily administration of 15γ of flavin promotes growth and restores the hair. The flavin is given in the free form; apparently the rat is capable of converting it into flavin phosphate. In fact some evidence has been presented showing that this may take place in the intestines (Verzàr, 1936).[25] It may be presumed provisionally that the necessity for flavin in the diet is to maintain the supplies of the yellow enzyme. But it is still possible that it has a function in the free state as there are suggestions in the literature that it can act as a catalyst in relation to certain dehydrogenase systems: the matter requires further investigation. It is to be noted that overdosage of some compounds allied to the flavins may result in the appearance of toxic symptoms, for Kuhn and Boulanger (1936)[26] found that with rats isoalloxazines, particularly the 9 phenyl derivative, were toxic.

Vitamin B_6.

The isolation of crystalline flavin from vitamin B_2 concentrates elucidated to some extent the conflicting results of different workers obtained in the study of the antidermatitis factor and raised the question of its possible multiple character. For, in 1930 Chick and Copping[27] and Roscoe[28] published data suggesting that the nature of vitamin B_2 was probably more complicated than previously supposed. These workers presented evidence for the existence of a factor, termed by them "Factor Y" of a stability to heat and alkali greater than vitamin B_2. The importance of this observation was more fully recognised when Kuhn, György and Wagner-Jauregg[10] found that flavin alone was incapable of

curing rat dermatitis and promoting growth. The missing essential constituent could be provided by the addition to the diet of an acid charcoal adsorbate of yeast, a source of vitamin B_4. The heat stability and curative action towards rat pellagra led György to call the missing factor, vitamin B_6.[29] Chick and her colleagues[30] and György[29] are now agreed that vitamin B_6 and factor Y are identical and together with flavin constitute what was previously known as vitamin B_2. It is important to remember that at present the term vitamin B_6 connotes an impure concentrate which may contain other additional factors.

The effect of a deficiency of vitamin B_6 has been amply demonstrated by feeding rats on synthetic diets supplemented with vitamin B_1 and flavin in their pure crystalline forms. From such experiments it has been found that typical rat dermatitis, previously attributed to lack of vitamin B_2 occurs only when the fraction of the vitamin B_2 complex termed vitamin B_6 is absent from the diet. The deficiency creates skin lesions of florid nature and of symmetrical distribution. Initially they manifest themselves by a soreness at the nose, eyes and ears and a redness and swelling of the feet. With time they accentuate: the pinnæ are thickened and encrusted; there is a marked œdematous appearance of the mouth and the paws; the latter are usually scabby. Simultaneously these symptoms are accompanied by gastro-intestinal disturbances.* The urine may be reduced in volume and be highly pigmented containing porphyrin. Such symptoms are rapidly alleviated by the administration of Peters' eluate, the decomposed lead precipitate from yeast extracts or by liver extracts.[31]

From time to time attempts have been made to correlate the symptoms of vitamin B_2 deficiency with lesions other than dermatitis. It has been proposed to term vitamin B_6 the rat acrodynia factor[32] on the basis of a similarity of the dermatitis observed in rats to the condition of the hands, etc., seen in children suffering from Pink disease. Such a superficial resemblance may be visualised but neglects the other aspects of the clinical picture of Pink disease. In the opinion of one of the authors, the symptoms do not resemble in detail Pink disease in children.† It has also been suggested that vitamin B_2 evokes cataract in rats.[33,34] At present in view of the conflicting evidence which may be in part due to the use of different diets, it is impossible to reach a definite conclusion. In our laboratory no instances of cataract have so far been observed in young rats on a diet deficient only in vitamin B_6.

In Table II is summarised the distribution of flavin and vitamin B_6:

TABLE II.

Distribution of flavin and vitamin B_6.[1,2,5,29,31,50,51]

Source	Flavin	Vitamin B_6
Yeast ..	+ + +	+ + +
Liver ..	+ + +	+ + +
Fish muscle ..	small amount	+ + +
Egg white ..	+ + +	small amount
Kidney ..	+ + +	?
Milk ..	+ + +	+ + +
Muscle ..	+	
Suprarenals ..	+	
Corpus Luteum ..	+	
Brain ..	+	
Retina of eye ..	+	

It will be observed that whereas flavin and vitamin B_6 are somewhat equally distributed in liver and yeast, they are unequally so in the egg white, fish muscle and other tissues. White of egg contains mainly ovoflavin with little vitamin B_6 a fact which accounts for the early observation of Chick on egg white as a source of vitamin B_2.

Of the chemical nature of vitamin B_6 little is known. It is not precipitated by the salts of Pb, Hg or Ag; it is precipitated by phosphotungstic acid, is adsorbed on Fullers' earth at acid pH, inactivated by benzoylation, untouched by nitrous acid, migrates towards the cathode on electrodialysis. It may be a basic substance containing an OH group.[35]

VITAMIN B_4.

This factor is now not so well defined an entity as the original methods of testing

* This is suggested by the occurrence of diarrhœa and abnormal appearance of the gut on post-mortem examination.

† The symptoms resulting from a deficiency of Reader's vitamin B_4 did resemble Pink disease, but see below.

for it have broken down. Originally Reader (1929)[35] described a third rat factor under the name of vitamin B_4 which promoted the growth of young rats on a diet supplemented by autoclaved marmite (vitamin B_2) and a preparation of vitamin B_1 free from vitamin B_4.‡ Later owing to the difficulties of test, the development of a method using adult rats was undertaken. On the same diet the rats showed peculiar symptoms of redness and swelling of the paws together with ataxia. Endeavours to substantiate these findings have failed, the red swollen paws being observed in only 1-2 per cent. of the experimental animals. Ataxic symptoms when present could be cured by the administration of 3-5 units of vitamin B_1 in the form of a crude concentrate or pure crystalline form.[37] The conflicting results may be explained in the future when we possess a pure preparation of vitamin B_6. The difficulty of producing vitamin B_4 deficiency in the rat has been indicated by Kline, Elvehjem and Hart[38] who consider that careful purification of the dietary constituents and the use of highly potent concentrates of the other factors of the vitamin B complex are essential for success. These workers succeeded in reproducing the ataxic symptoms without the red swollen paws, and found that pea nuts alleviated the condition.§

Pellagra.—Pellagra is a disease characterised by gastro-intestinal disorders, nervous disturbances and extensive skin eruptions, occurring in different parts of the world, particularly maize-eating countries. The nature of the causal agent is still a matter of dispute although of recent times, it has generally been held to be of dietary origin. The work of Goldberger and his colleagues[40] in America and Wilson[41] in Egypt laid the foundations for this hypothesis. The early view of Wilson (which he himself still maintains) that a shortage of protein of high biological value was responsible for the condition gave place to one of vitamin deficiency. In exploring the curative properties of different foodstuffs Goldberger reached results difficult to reconcile with the assumption that adequate protein in the diet cured pellagra. A protein such as casein showed no curative action whilst an acid extract of yeast containing little protein matter was effective in curing the disease. These results led to the postulation of the P-P factor, a deficiency of which caused the onset of the pellagrous condition. The close resemblance of the symptoms seen in the rat deprived of vitamin B_2 to those in pellagrins suggested a similarity if not identity in nature in the P-P factor and vitamin B_2. Aykroyd and Roscoe[42] pointed out that the distribution in foodstuffs of vitamin B_2 and the P-P factor was similar. Experimental black tongue in dogs, a pellagrous condition, is reproducible on pellagrin diets and is cured by a factor which, like vitamin B_2, is thermostable: yet two features, associated with human pellagra, a prevalence in maize-eating countries and the detrimental effect of sunlight have still to be correlated with these results. So far it has not been completely proved that sunlight stimulates rat dermatitis. (Hogan[43] has produced a form of dermatitis by exposure of rats to ultraviolet light.) Even more difficult to reconcile with the view that vitamin B_2 and the P-P factor are one and the same is the finding of Birch, György and Harris (1935),[44] that maize and the diets of pellagrins are rich in vitamin B_6. Dogs are found to develop black tongue when fed on a Goldberger maize diet containing large amounts of vitamin B_6. It is therefore concluded that vitamin B_6 is a factor distinct from the P-P factor and the anti-black tongue factor although the two latter may be identical. That flavin is not the P-P factor has been demonstrated by Dann who observed no improvement in pellagrins on administration of the compound. Despite such evidence indicating that human pellagra, rat dermatitis and black tongue in dogs arise from deficiency of different entities, it is also possible that one or more factors are operative in a given condition. The cures of children suffering from stomatitis by feeding such sources of vitamin B_2 as yeast and milk have been made by Aykroyd and Krishnan[45] who discuss the possibility of pellagra arising from a deficiency of more than one factor.

Vitamin B_2 and Anæmia.—The co-existence of vitamin B_2 and the extrinsic factor of pernicious anæmia in liver, liver extracts, marmite and yeast led Castle and Strauss

‡ Probably flavin was the factor under test.

§ It has recently been reported by McHenry[89] that vitamin B_4 is possibly choline, but his experiments require confirmation.

(1932)[46] to suggest that vitamin B_2 was probably the extrinsic factor upon which the intrinsic factor acted. This view has not been confirmed. Wills (1933)[47] incubated purified extracts of vitamin B_2 with the intrinsic factor and found no improvement in cases of anæmia treated with the digestion mixture. More recently Wilkinson (1935)[48] showed that flavin was ineffective in anæmia. The isolation of the anti-hæmatopoietic factor by Dakin and West (1935)[49] should throw light upon the possible relation of the anti-anæmia factor to vitamin B_2 complex.

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