LXXXIV. THE WATER-SOLUBLE B-VITAMINS.
IV. THE COMPONENTS OF VITAMIN B_2.

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Note. Vitamin B_2 may be defined as the heat-stable constituent of the vitamin B complex which must be added to diets containing all other dietary essentials, including the antineuritic vitamin B_1, in order to maintain growth and health in the rat and prevent occurrence of dermatitis ("rat pellagra").

THE recent isolation of lyochromes, water-soluble pigments with characteristic yellow green fluorescence, by Ellinger and Koschara [1933] and by Kuhn *et al.* [1933, 1, 2, 3] and the discovery that these pigments are present in materials possessing the biological attributes of vitamin B_2 have given a new impetus to the study of this vitamin. A series of such pigments, having certain well-defined physical and chemical properties in common, has been isolated in pure form from many substances rich in vitamin B_2, *viz.* whey, egg-white, yeast, liver, kidney [Ellinger and Koschara, 1933; Kuhn *et al.*, 1933, 1, 2, 3; Warburg and Christian, 1932; 1933; Karrer *et al.*, 1934].

At first some of these pigments, when administered even in very small doses, were thought to possess the whole nutritive properties of vitamin B_2. Later, however, as purer specimens were available for testing, it became apparent that neither ovoflavin nor lactoflavin nor hepatoflavin [Kuhn *et al.*, 1933, 2, 3; Kuhn, Rudy and Wagner-Jauregg, 1933; György *et al.*, 1933; Euler *et al.*, 1934], when added with vitamin B_1 to a "-B" diet, could supply the full function of vitamin B_2, but that some further supplement was necessary; in other words, that what had hitherto been regarded as vitamin B_2 contained at least two constituents. In these experiments the vitamin B_1 was supplied as an alcoholic cereal extract [György *et al.*, 1934] or as an adsorbate on acid clay prepared from rice polishings by Jansen and Donath's method [Euler, Karrer and Alder, 1934].

The missing supplement for lactoflavin or ovoflavin was provided by the addition to the diet of a yeast extract, from which the B-vitamins or lyochromes had been adsorbed by treatment with fuller's earth [Kuhn *et al.*, 1933, 2; György *et al.*, 1933; Euler, Karrer and Malmberg, 1934] or when vitamin B_1 was supplied as a concentrate made from baker's yeast by the Peters process [György *et al.*, 1934]. The latter observation at first led György and his co-workers to the conclusion that the heat-labile "vitamin B_4" [Reader, 1929; 1930, 1] was the missing factor. "Vitamin B_4" is stated to accompany vitamin B_1 to the end stage of the Peters process, as ordinarily carried out [Reader, 1930, 2]. The conclusion that "vitamin B_4" was involved has, however, recently been abandoned by György [1934] in favour of the idea that the supplement needed by flavin, which is present in the Peters preparation, is a new heat-stable component of the vitamin B complex. This new vitamin György [1934] has called "vitamin

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B_6" and has suggested that it may be identical with the "factor Y" described by Chick and Copping [1930, 2] and Roscoe [1930]. This vitamin B_6, together with the flavin, forms a combination having the full biological action formerly attributed to vitamin B_2.

The above forms a very brief summary of work published in a large number of small papers during the last two years. In most of these only scanty details were given of the animal tests which formed the basis of the conclusions drawn as to the relation of the flavins to vitamin B_2. The subject seemed to us to need fuller investigation and the following experiments were begun about a year ago, in the hope of elucidating the nutritive properties of flavin and its relation to the vitamin B complex, not only for maintaining growth, but possibly also for preventing and curing dermatitis in rats.

The lyochromes investigated by us included a specimen of pure lactoflavin D prepared from whey (analysis: C, 52·75, 52·69; H, 5·70, 5·84; N, 14·43, 14·38 %; M.P. 269–273° (uncorr.)) kindly provided by Prof. Ph. Ellinger, and one of pure hepatoflavin from liver (analysis: C, 55·01, 54·85; H, 5·53, 5·65; N, 14·33, 14·32 %; M.P. 275–280°) placed at our disposal by Dr Sydney Smith. These specimens agreed well, both in analysis and melting-point, with those isolated respectively by Kuhn *et al.* [1933, 3] (lactoflavin) and by Karrer *et al.* [1934] (hepatoflavin). We wish here gratefully to acknowledge these gifts. Without them this work would have been impossible.

METHODS.

Diets. The tests for vitamin B_2 were carried out by the methods usual in this laboratory [Chick and Roscoe, 1928]. Young rats, when weaned, received the usual "-B" diet (caseinogen 100, rice starch 300, cottonseed oil 75, salt mixture (McCollum's No. 185) 25, water 500). The diet was heated in a steamer for 3 hours at 100°, in order to cook the starch grains, so that "refection" might be prevented [see Roscoe, 1927]. Vitamins A, D and B_1 were administered separately to each rat daily by capillary pipette, A and D as 0·08 g. cod-liver oil and B_1 as 0·1 ml. ($\equiv$0·6 g. original yeast, dry weight) of a Peters concentrate prepared from brewer's yeast [Chick and Roscoe, 1929].

We have not found any advantage in the elaborate purification of the caseinogen previously adopted [see also Roscoe, 1933] and for most of the present work a commercial sample of "light white casein" was used. A few comparative tests were made with similar "-B" diets containing purified caseinogen, but again no difference was detected either in the extent to which growth was checked or in the incidence of dermatitis.

In some of the diets the rice starch was replaced by an impure sample of maize sugar (containing 85 % dextrose, 1 % ash and *ca.* 14 % maltose, dextrins and other impurities), such as is used in this country in the manufacture of beer. This change was made in order to see if a maize derivative (by analogy with human pellagra) might prove conducive to regular development of the specific skin disorders which at the time were occurring only rarely in our young rats on diets deficient in vitamin B_2. The use of sucrose in the basal diet in place of starch was found conducive to the development of dermatitis in rats deprived of vitamin B_2 by Hogan and Richardson [1934].

Growth tests. Growth frequently ceased immediately from the time the rats received the experimental basal diet; in other cases there was very slow but steady increase in weight for several weeks. The difference appeared to depend on the reserves of B-vitamins with which the young rats were endowed and it has been noted that rats from our colony have appeared to be less sensitive to lack of vitamin B_2 since a generous allowance of dried yeast has been included in the breeding diet. This change was introduced to produce young rats in a more satisfactory condition for work on vitamins A and D. We have therefore tried the effect, with good results, of limiting the B-vitamins, especially vitamin B_2, in the diet of the mothers of litters intended for vitamin B_2 work. No yeast is given during lactation, the mothers' diet consisting of mixed

cereals, milk, raw cabbage and carrot, for the first 2 weeks, and for the third week of the "-B" experimental diet supplemented with vitamin B_1 as Jansen and Donath's acid clay adsorbate from rice polishings. If the weight increase of the litter appeared to be too severely checked, milk was given in addition during the latter period and the weaning postponed for a few days, until the weight of the young rats was 30–40 g. This procedure has been successful in producing rats much more sensitive to vitamin B_2 deficiency, showing more regular cessation of growth and a more regular and quicker development of skin disorders.

When the body weight was stationary the daily doses of flavin were administered, with and without the various supplements to be tested, and growth was observed for 3 or 4 weeks or longer.

Table I. *Incidence of dermatitis in young rats receiving a diet deprived of vitamin B_2 from the time of weaning, the mothers being also deprived of this vitamin during the last week of lactation.*

Vitamin B_1 administered separately to each rat as 0·1 ml. ($\equiv$0·5 g. yeast dry weight) of a "Peters concentrate" prepared from brewer's yeast.

(*a*) signifies the florid type of dermatitis ("rat pellagra"). ± signifies slight or incipient.
(*b*) ,, the more generalised type. + ,, definite.
0 ,, no symptoms. + + ,, severe.

Result after 9 weeks on the experimental diet.

Litter	Carbohydrate in basal diet	Rat No.	Body weight g.	Incidence of dermatitis Type (*a*)	Incidence of dermatitis Type (*b*)	General condition
23	Maize sugar	211	42	+ +	+ +	Very bad, rat died
	,,	214	56	±	0	Good
	,,	215	59	±	+ +	Fair
	,,	218	55	±	0	Good
	Rice starch	212	48	0	±	Fair
	,,	213	41	0	+ +	Very poor
	,,	216	48	+	+ +	Poor
	,,	217	41	0	±	Good
24	Maize sugar	219	72	±	0	Good
	,,	223	67	0	+	Good
	,,	224	57	0	±	Fair
	,,	226	83	0	+	Good
	Rice starch	220	59	0	+ +	Poor
	,,	221	61	0	+ +	Poor
	,,	222	53	±	±	Good
	,,	225	45	+ +	+	Poor

Cure of dermatitis. The irregularity in the occurrence of dermatitis in rats deprived of vitamin B_2, for which no adequate explanation has been forthcoming, has hitherto been a hindrance to the study of this syndrome [see Roscoe, 1933; Hogan and Richardson, 1932]. We have now concluded that differences in the reserves of vitamin B_2 possessed by the young animals at the beginning of the experiment are the principal cause of this uncertainty. The substitution of impure maize sugar for starch in the basal diet has also seemed to encourage the regular incidence of dermatitis.

The skin disorders which have been observed by us in rats suffering from deficiency of vitamin B_2 (using this term in the sense defined at the beginning of this paper) have shown the following two types [Chick and Roscoe, 1928, p. 795; Chick and Copping, 1930, 1, p. 933; see also György, 1934]:

(*a*) A florid dermatitis, roughly symmetrical, in which there is redness, swelling and oedema of the skin of the extremities (ears, digits of the fore and hind paws) and sometimes also of the nose and corners of the mouth; this may spread later over the limbs and trunk with cracking and desquamation of the skin. This type of dermatitis corresponds to that usually called "rat pellagra" in the literature. It has recently been described as the "specific type" of dermatitis by György [1934].

(*b*) A general affection of the skin, without swelling or inflammation, in which there is shedding of the hair, the remaining locks becoming matted and bald patches sometimes occurring later, especially over the head and face and on the anterior surface of the fore paws. The eyes acquire a "spectacled" appearance from loss of the eyelashes and surrounding hair; the eyes are usually sunken and a bloody serous fluid exudes from the sore swollen eyelids, which may be stuck together, and also from the orifice of the nostrils. The urine is scanty and concentrated and dribbling occurs with consequent soiling and staining of the abdomen. These appearances agree well with the description given by György of his "non-specific" skin changes.

In the succeeding pages these two kinds of skin affection are referred to as types (*a*) and (*b*) respectively.

In our experience young rats receiving a diet deprived of vitamin B_2 (in the old sense) may develop either the (*a*) or (*b*) type of dermatitis or a combination of both sets of symptoms. The (*b*) condition is the more usual and generally occurs sooner in young rats deprived of vitamin B_2. The animals affected remain much undersized and appear to suffer constitutionally to a severe degree from the vitamin deprivation. When the florid (*a*) symptoms develop, they usually occur later in rats whose general condition is stronger; sometimes they are superposed on the (*b*) symptoms in animals who survive for some time after the latter have developed.

This generalisation is illustrated in Table I, which gives the history of two litters of rats, which developed dermatitis on a "-B_2" ration. Among 16 rats maintained for 9 weeks after weaning on the experimental diet, the generalised (*b*) skin symptoms were present in 13 and to a severe degree in 6 of these, of which 1 died. At the same date only 8 rats showed signs of "florid" (*a*) dermatitis and in 5 of these it was only slight or incipient and the general condition of the rats was relatively good.

Results.

The effect of flavin was tested on rats whose growth had failed on a diet deprived of vitamin B_2 as well as on those suffering in addition from either form of skin trouble and on some in which there was a syndrome combined of both types.

Effect on growth.

The results collected in Table II show that doses of pure lactoflavin and hepatoflavin, of 20–50γ daily, certainly stimulated growth to a small extent (average weekly increase in weight 5–8 g.), in comparison with the previous performance of the rats on the "-B_2" diet. Smaller doses, 6–12γ, of hepatoflavin were less effective; there was no appreciable increase of weight over a period of 4–6 weeks in the case of 2 rats receiving 6γ, and in 5 out of 8 rats receiving 12γ daily. When, however, doses of 20–40γ flavin were supplemented by a small dose of an autoclaved yeast extract (A) (equivalent to 0·5 to 1·0 g. dried yeast) which showed no vitamin B_2 activity after two successive treatments with fuller's earth at p_H 1·0, a rate of growth was attained which approached the normal standard (12–14 g. weekly increase in weight). A small amount of a yeast extract (B) (equivalent to 0·5 g. dried yeast), in which all the known B-vitamins had been destroyed by autoclaving at 120° for 5 hours at p_H 9 (*ca.*), supplemented flavin equally well, and normal growth was also obtained. With smaller doses of hepatoflavin, the supplements appeared to be less effective and the degree of growth seemed to be limited by the size of the flavin dose (see Table II).

The minimum rat day dose of hepatoflavin which was necessary for "normal growth" was 12–20γ, a value somewhat higher than that found by other workers. György [1935] found 7–10γ lactoflavin adequate as a rat day dose; Euler, Karrer and Malmberg [1934] 5–10γ, but their criterion for growth was lower than that adopted by us.

Table II. *Effect of lactoflavin and hepatoflavin on rats whose growth had ceased on a diet deprived of vitamin B_2.*

Vitamin B_1 given as 0·1 ml. daily (≡ 0·5 g. dry yeast) of a concentrate prepared from brewer's yeast by Peters's method (see Chick and Roscoe [1929]; method modified according to Kinnersley *et al.* [1933]).

Supplement A. Extract of brewer's yeast deprived of B-vitamins by treatment with fuller's earth (dose ≡ 0·5 g. dry yeast).

Supplement B. Yeast extract deprived of B-vitamins by autoclaving 5 hours at 120° at p_H 9 (dose ≡ 0·5 g. dry yeast).

		Average weekly increase in weight over a period of 4–6 weeks				
		(*a*) Flavin		(*b*) Flavin + supplement		
Flavin	Daily dose γ	g.	No. of rats	g.	No. of rats	Nature of supplement
Lactoflavin	10	5·1	2	12·3	2	A 1 rat; B 1 rat
,,	20	5·3	3	11·7	2	,, ,,
Hepatoflavin	6	2·6	2	5·1	2	A
				7·1	3	B
,,	12–16	4·2	6	8·9	4	A
				10·0	2	B
,,	20–50	7·8	8	13·8	5	A
				13·8	5	B
				(*c*) Supplement only*		
				1·1	7	A

* Time of observation 2–10 weeks.

Effect on dermatitis.

Lactoflavin was found to be without any curative effect upon florid dermatitis ("rat pellagra"), a result confirming that obtained by György [1934]. On the contrary, the symptoms were frequently developed after the administration of flavin to rats whose growth had ceased on the "-B_2" diet, but which showed no sign of dermatitis. This was observed in 2 rats receiving 10–20γ of lactoflavin and in 6 rats receiving 12–50γ hepatoflavin (see Table III, rats 98, 141).

Of 9 rats showing the (*b*) group of symptoms in various degrees of severity, the administration of flavin was without any effect in six cases, the result was doubtful in one case, there was slight improvement in one and a most dramatic cure was observed in one severe case (rat 147, Table III) after administration of 24γ hepatoflavin daily. Only in 1 rat showing no skin affection did the (*b*) symptoms develop in slight degree after administration of flavin and in 1 rat slight symptoms became more aggravated. These 2 rats received 20 and 10γ lactoflavin respectively daily.

With one or two exceptions therefore, in these experiments, flavin showed no curative effects on either of the two types of skin disorder.[1] When, however, supplement A or B was given in addition, swift and regular cure of the skin condition accompanied the resumption of growth. There was little difference whether the skin lesions were of the (*a*) or (*b*) type, or a combination of both. Some typical histories of such cures, which included observations on 22 rats suffering from type (*b*) and on 7 rats showing the florid type (*a*), are summarised in Table III.

[1] See footnote, p. 730.

Table III. *Effect of pure lactoflavin with and without supplements on the dermatitis developed in young rats deprived of vitamin B_2.*

Supplement A = Autoclaved yeast extract, treated with fuller's earth at p_H 1·0 to remove vitamin B_2.
" AC = Supplement A after dialysis.
" AD = Dialysate from supplement A.
" B = Yeast extract, autoclaved at 120° for 5 hours at p_H 9–10 to destroy all known B-vitamins.

Diet St. contained carbohydrate as rice starch (60 % on dry wt.); diet MS as maize sugar (60 %).
(For descriptions of (*a*) and (*b*) types of skin affection, see pp. 724, 725).

Curative material								Body weight			
	Daily dose γ	Supplement	Dose in terms of original yeast (dry wt.) g.	Rat No.	Diet	Time previously on "-B_2" diet weeks	Skin symptoms, and general condition at beginning of cure	At beginning of cure g.	Average weekly increase g.	Duration of cure weeks	Result of treatment
Lactoflavin	20	None	—	98	MS	3	None	47	3·9	10	Florid (*a*) dermatitis developed on hind and fore paws and on nose
"	20	B	0·5	98	MS	—	As in column 12	86	11·5	4	Cure. Skin condition good, dermatitis healed, fur thick but greying
"	20	None	—	128	MS	4	Slight (*b*) type symptoms. Fur poor, greasy and stained; eyes, conjunctivitis. General condition poor	32	5·6	5	Skin condition more severe, in spite of some increase in weight
"	20	A	0·5	128	MS	—	As in column 12	60	9·8	4	Incomplete cure. Skin and fur almost normal, eyes slightly sunken and secreting
Hepatoflavin	12	None	—	141	MS	13	Skin symptoms slight. Eyes sunken and secreting. General condition not good	97	2·0	4	Skin condition deteriorated. Ear tips thickened, paws showing incipient florid (*a*) dermatitis. Wrists stained, eyes sunken and secreting
"	12	AC	1·0	141	MS	—	As in column 12	105	−2·0	3	Condition much worse in every particular. Severe florid (*a*) dermatitis
"	12	AD	1·0	141	MS	—	As in column 12	99	6·2	(4)	Cure. Fur much improved, dermatitis healing
"	12	B	0·5	202	St.	4	Skin symptoms (*b*) type. Fur matted and greasy; bald patches on neck. Condition miserable	36	12·0	4	Rapid cure. Skin and fur became normal in 2–3 weeks, steady increase in weight
"	12	None	—	181	MS	9	Fur scanty, animal almost bald, eyes very sunken, abdomen stained. Florid (*a*) dermatitis on fore and hind paws	40	2·7	4	Type (*b*) symptoms in eyes, fur improved, florid (*a*) dermatitis present on paws
"	12	A	0·5	181	MS	—	As in column 12	51	11·7	4	Cure. Florid (*a*) dermatitis healed
"	24	None	—	147	St.	18	Very severe symptoms of (*b*) type. Fur matted and greasy; abdomen much stained with highly pigmented urine; very severe conjunctivitis. Condition wretched	68	10·3	3	Good cure. Great improvement in eyes and fur in 5 days; staining gone in 2–3 weeks

Influence of the composition of the basal diet upon the development and cure of dermatitis.

(*a*) *Type of carbohydrate.* Whether addition of flavin hastened or stimulated the development of "florid" dermatitis ("rat pellagra") seemed to be related to the nature of the carbohydrate in the "-B_2" basal diet. Among 24 rats on which tests were made with daily doses of unsupplemented flavin, 12 were receiving their carbohydrate in the form of rice starch and 12 as maize sugar. The 8 rats which developed florid dermatitis after dosing with flavin included 7 of the latter group and only 1 of the former.

The administration of flavin, as contained in a small daily dose (5 g.) of raw egg-white, to 5 young rats which had ceased to grow on a "-B_2" ration, while showing no skin affection, caused development of a slight degree of florid dermatitis in 3 receiving the maize sugar diet but restored growth without any effect on the skin in 2 receiving the rice starch diet.

(*b*) *Source and dose of vitamin B_1.* A series of experiments in many ways similar to those described above has recently been carried out by György at Cambridge [György, 1935] and these we have been permitted to observe. In György's experiments the florid type of dermatitis was regularly developed on a "-B_2" diet, in which vitamin B_1 was given in pure crystalline form together with flavin, and was as regularly cured when the vitamin B_1 dose was changed to a Peters concentrate from baker's yeast, given in a relatively large dose (daily rat dose 1–2 ml. equivalent to 10–20 g. fresh, or *ca.* 3–6 g. dry, yeast).

We, on the other hand, had never detected the slightest curative or preventive effect for rat dermatitis in Peters's vitamin B_1 concentrate prepared in our laboratory from brewer's yeast (see Chick and Roscoe [1929], method modified according to Kinnersley *et al.* [1933]), but our rat daily dose (always controlled in separate trials for adequacy in vitamin B_1) was much smaller when reckoned on the basis of the original yeast from which the concentrate was prepared (*i.e.* equivalent to 0·6 g. dry yeast). For this reason alone it might be assumed to carry only about 1/5–1/10 of the adherent yeast "impurity" given in György's dose. It therefore seemed probable that Peters's concentrate, as used by him, might carry an adequate supply of the necessary supplement for flavin. By the kind co-operation of Dr György we have been enabled to test this assumption.

Two rats, 169 and 205, were chosen which had ceased to grow on our "-B_2" diet; both showed severe symptoms of the type (*b*) and one (169) of the florid dermatitis, type (*a*), also. In place of their previous vitamin B_1 addition they received the dose advised by György of his own Peters's vitamin B_1 concentrate (2 ml. $\equiv$ *ca.* 6 g. dry yeast), together with 12γ hepatoflavin. In both cases growth was immediately restored (average weekly increase in weight 18 and 17 g. respectively) and there was swift and complete cure of the skin lesions. For comparison, the effect of a much increased dose (0·3 ml. $\equiv$ 1·8 g. dry yeast) of our Peters's vitamin B_1 preparation, together with 12γ hepatoflavin was tried on 2 litter-mates (rats 167 and 203) of the above rats, which showed a comparable condition of vitamin B_2 deficiency. The effect on growth and dermatitis was very slight in comparison with that observed in rats 169 and 205 and about comparable with that observed when a much reduced dose was given (0·5 ml. $\equiv$ *ca.* 1·5 g. dry yeast) of György's preparation (rat 203). These results are summarised in Table IV.

György [1934; 1935] has interpreted these results as showing a separate specific action for the two types of dermatitis to be possessed by the two con-

Table IV. *Influence of vitamin B_1 ration.*

Diet St. contained carbohydrate as rice starch (60 % on dry wt.); diet MS as maize sugar (60 %).
(For descriptions of (*a*) and (*b*) types of skin affection, see pp. 724, 725.)

Curative material (vitamin B_1 dose)								Body weight			
	Daily dose γ	Supplement	Dose in terms of original yeast (dry wt.) g.	Rat No.	Diet	Time previously on "-B_2" diet weeks	Skin symptoms and general condition at beginning of cure	At beginning of cure g.	Average weekly increase g.	Duration of cure weeks	Result of treatment
Hepatoflavin	12	György's B_1 concentrate (Peters's method)	5·0	169	St.	9	Florid (*a*) dermatitis on ears, hind and fore paws. No conjunctivitis or staining of fur. Condition poor	57	17·7	3	Rapid cure. Marked clearance of symptoms in 10 days
,,	12	,,	5·0	205	St.	8	Skin affection (*b*) type. Fur matted and stained on abdomen	35	11·0	3	Rapid cure. Great improvement in 10 days
,,	12–24	Lister B_1 concentrate	0·6	207	MS	7	Very severe (*b*) type. Fur stained and matted, eyes sunken and secreting, condition miserable	41	2·0	4	Improvement in (*b*) symptoms but eyes still sunken. Development of florid (*a*) dermatitis on ears, fore and hind paws; skin dry, scurfy and cracking
,,	12–24	György's B_1 concentrate	1·0	207	MS	—	As in column 12	47	6·2	3	Cure almost complete
,,	12	Lister B_1 concentrate	1·8	167	MS	9	Combined (*a*) and (*b*) types. Ears and paws inflamed, fur stained and matted	52	9·0	4	Incomplete cure. Ears and hind paws not quite normal. Fur much improved
,,	12	,,	1·8	203	St.	8	Combined (*a*) and (*b*) types. Eyes affected, fur greasy and stained, ear tips inflamed. Condition miserable	35	6·3	4	Incomplete cure. Coat improved but paws scurfy. Condition still poor
,,	12	György's B_1 concentrate	5·0	203	St.	—	As in column 12	60	11·0	3	Skin condition improved

stituents of vitamin B_2. Thus "vitamin B_6" (as contained in vitamin B_1 preparations made from yeast by the Peters process) has a definite action in curing the florid (or "specific") dermatitis and in counteracting the influence of flavin in producing this type. Flavin, on the other hand, possesses a definite curative action for the generalised "non-specific" dermatitis. To maintain steady growth both constituents are required.

We also have found the addition of flavin to a diet deficient in vitamin B_2 frequently conducive to the development of the florid type of dermatitis or to its aggravation when present, and we have found further that this action in some way depends on the type of carbohydrate contained in the basal diet. We conclude, however, that in combination with some dialysable, thermo- and alkali-stable substance contained in a yeast extract freed from all known B-vitamins, lacto- or hepato-flavin possesses curative properties for all forms of dermatitis and for the restoration of growth. The supplementary substance derived from yeast would appear to be absent from, or present in only negligible amount in, our Peters's vitamin B_1 concentrate and to be present in relative abundance in György's preparation. It is not possible to state whether this difference is to be explained by the differences in origin and method of preparation of the two products, ours from brewer's and his from baker's yeast, or whether it may not be due to the much larger dose given in his experiments, but the latter explanation appears at least probable.

We have observed the curative action recorded by György of unsupplemented flavin for the "non-specific" (*b*) skin symptoms, but rarely (Table III, rat 147), but we have noticed a definite effect in restoring growth which appears to be proportional to the dose of flavin given.[1]

In our trials with the supplementary material in the absence of flavin, we have obtained neither restoration of growth (see Table II *c*) nor consistent cure of either type of dermatitis. The action of a daily dose of supplement A (equivalent to 0·5 or 1·0 g. dry yeast) was tested over periods varying from 2 to 10 weeks on 7 rats showing skin affections in varying degree. Six of these rats showed the (*b*) type; in 1 of these the symptoms remained unchanged and in 5 definitely progressed. The (*a*) florid type was present to a slight degree in 5 of the rats and absent from 2; in 1 of the latter it developed and among the former the symptoms became worse in 1, remained unchanged in 2 and cleared up in 2.

Previous work by two of the present authors (undertaken to investigate the heat- and alkali-stability of vitamin B_2) showed complete failure of a yeast extract, after autoclaving at 120° for 5 hours at p_H 9·0 (*i.e.* supplement B), to influence dermatitis in rats deprived of vitamin B_2. In almost all the cases treated, the skin lesions were of the (*b*) type [Chick and Copping, 1930, 1; see also Roscoe, 1933]. One case is, however, quoted [Chick and Copping, 1930, 1, p. 934] in which the preparation showed a curative action for the florid type of dermatitis.

[1] *Note added February 26th, 1935.* Since the above was written we have observed more frequently a curative action of unsupplemented hepatoflavin for the (*b*) type of skin symptoms developed in rats deprived of vitamin B_2. Six rats, of body wt. 39–89 g., showing the (*b*) symptoms (4 in a severe degree) received daily 12 hepatoflavin; all showed some improvement, and 4 were completely cured, with a (subnormal) stimulation of growth. In 3 of the above rats the (*a*) florid type of dermatitis developed during this period.

When the supplementary material alone (vitamin B_6) was given in similar cases, the condition always deteriorated, nor have we observed any consistent curative action of vitamin B_6 for the florid (*a*) type when this was present, unless flavin was given also.

The above results thus contain suggestions that the two constituents of vitamin B_2 may sometimes function separately in the prevention and cure of the two different types of skin disorder, *viz.* flavin for the (*b*) type and the supplementary material for the (*a*) type. To this extent our conclusion confirms that arrived at by Dr György, but the general trend of our whole work indicates that for successful treatment of either condition, or of both when present together, and for maintenance or restoration of growth a combination of both the constituents of vitamin B_2 is necessary. All the above trials were, however, of the curative type and have the defects of this kind of experiment. It is probable that the relation between the two types of skin disorder and the two constituents of vitamin B_2 could be more satisfactorily investigated in prophylactic experiments made to observe the type of skin lesions developed when either flavin or the supplementary material is alone added to the basal "-B_2" diet from the time of weaning. Such experiments are now in progress.

Relation of the supplementary substance to the "factor Y" of Chick and Copping [1930, 2].

The facts recorded in the present paper may be summarised in the statements:

(*a*) that flavin is one constituent of vitamin B_2, as previously defined (p. 722), and

(*b*) that the second supplementary constituent is a water-soluble, dialysable, heat- and alkali-stable substance contained in yeast extract.

Vitamin B_2 as contained in natural foods (yeast, whey, liver, egg-yolk) would appear to be an association of these two constituents. In egg-white, however, the supplementary substance appears to be present in relatively small amounts.

In previous observations [Chick and Copping, 1930, 2; Roscoe, 1930] on the growth of rats maintained over a long period on synthetic diets in which the vitamin B_2 was derived from egg-white, and vitamin B_1 given as a minimum dose of a Peters concentrate from yeast, or as Jansen and Donath's adsorbate from rice polishings, a gradual failure of growth always occurred. This could be checked by substituting for the egg-white another natural source of vitamin B_2 or by addition of a yeast extract freed from known B-vitamins by prolonged autoclaving in alkaline solution. The required supplement was present in yeast, liver and egg-yolk, but relatively deficient in egg-white; it was heat- and alkali-stable and was provisionally called "factor Y". Some small amount of this factor was evidently present in our Peters's vitamin B_1 concentrate from brewer's yeast, for when large doses were used they supplemented the action of the vitamin B_2 from egg-white. "Factor Y" would thus appear to be identical with György's vitamin B_6, the supplementary substance needed by flavins in order to exercise the full function hitherto attributed to a single vitamin B_2. Until, however, the biological activity of the flavins was discovered [Kuhn *et. al.*, 1933] the status of our "factor Y" could not be more precisely defined.

Properties of vitamin B_6. Vitamin B_6 or "factor Y" is the most heat- and alkali-stable constituent of the B-group of vitamins; it can resist prolonged autoclaving in alkaline solution (p_H 9·0), *e.g.* in supplement B. For further investigation supplement A was chosen as providing the purer material, but the work has not as yet progressed very far. The activity is found present in the dialysate after dialysis through a cellophane membrane and is not removed by precipitation with lead acetate either at p_H 4·0 or 8·0, in this property affording a contrast with flavin.

Nomenclature of vitamin B_2 and its constituents.

Elvehjem and Koehn [1934], on the basis of experiments with chickens, have suggested that the term vitamin B_2 be reserved for the dermatitis ("pellagra")-preventing factor of vitamin B_2-containing materials. They found that flavins prepared from a liver extract were inactive, but that the fraction remaining after these were adsorbed by fuller's earth was highly active, in "prevention of pellagra". The material they desire to call vitamin B_2 would appear to correspond with our vitamin B_6 or "factor Y". This, from the investigation described in the present paper, is clearly only one constituent of vitamin B_2 as previously understood, and requires to be associated with flavin in order to show the full activity of that vitamin. It seems to us that the use of the term "vitamin B_2" to signify either the non-flavin or the flavin constituent of what in the past was known as vitamin B_2 can only cause confusion in the literature of this subject. The custom adopted by workers concerned primarily with the chemistry of the flavins of alluding to these pigments as "vitamin B_2" adds to the confusion [*e.g.* Kuhn and Weygand, 1934].

We would suggest that the term vitamin B_2 be retained for the association of (i) flavin and (ii) supplementary substance, that (i) be known simply as flavin, since it has been identified with a substance of known chemical constitution, and that (ii) be called vitamin B_6 until such time as its chemical nature and identity are established.

Summary.

1. The results of experiments on rats described in this paper indicate that vitamin B_2, as previously defined, is composed of two constituents: (i) flavin, (ii) a supplementary substance contained in yeast extract.

2. The supplementary substance appears to be identical with the "factor Y" previously described by Chick and Copping [1930, 1], and with the "vitamin B_6" described by György [1934]. It is heat- and alkali-stable, can dialyse through a cellophane membrane, is not adsorbed by fuller's earth at p_H 1·0 or precipitated by lead acetate at p_H 8·0 or 4·0. It is present in small amount in the vitamin B_1 concentrate prepared from yeast by Peters's process and can be detected when large doses are given.

3. Pure hepatoflavin or lactoflavin, when added as a small daily dose (12–20γ) to a basal diet deprived of vitamin B_2, but containing vitamin B_1 (as a minimum daily dose of a concentrate prepared from brewer's yeast by Peters's process), was found to restore growth in rats to a small extent (increase of weight up to 6 g. weekly). In order to attain normal growth (10–12 g. weekly) a small daily dose of the supplementary material (equivalent to 0·5–1·0 g. dry yeast) was also necessary.

4. Flavin when given alone (in doses of 12–20γ daily) was found to be without effect upon the characteristic "florid" dermatitis, type (*a*), "rat pellagra", developed in young rats deprived of vitamin B_2; in some instances its administration appeared to encourage the development of this type of skin disorder in animals where it had not yet developed. In some animals suffering from the more generalised type of skin disorder, described as type (*b*) in this paper, flavin appeared to have a curative action; in others there was no effect.

5. The supplementary material, when given alone (in doses equivalent to 0·5–1·0 g. dry yeast), had no effect in restoring growth, while the (*b*) type of skin disorder, if present, progressed without any check. Upon the florid (*a*) type

of dermatitis, there appeared sometimes to be a slight curative effect, but the results were irregular and inconclusive.

6. When flavin and the supplementary material (in the above doses) were given together, there was a speedy cure of either the (*a*) or (*b*) type of skin disorder, or of both, in cases where there was a combined syndrome, and normal growth was immediately restored.

7. It is suggested, in order to prevent confusion in the literature of the subject, that the term *vitamin B_2* be retained to signify the two factors in association, that the one constituent be termed simply *flavin*, and the second, supplementary, substance be called *vitamin B_6*, as suggested by György [1934].

In conclusion we wish to express our thanks to Sir Charles Martin for his interest and kindly criticism and to Dr P. György for providing us with materials used for experiments summarised in Table IV, and for his friendly co-operation. Our thanks are also due to the Corn Products, Ltd. for supplying the information regarding the sample of maize sugar used in the basal diet. Our indebtedness to Dr Ph. Ellinger and Dr S. Smith for the samples of pure flavin has already been expressed.

Note on the influence of light upon vitamin B_2.

Pure flavin has been shown to be sensitive to visible light, the change being accompanied by loss of biological properties [György *et al.*, 1933]. Many observers in the past have reported destruction of vitamin B_2 in yeast and yeast extracts by exposure to ultra-violet radiation from a quartz mercury arc; and this method has been used to free yeast extracts from vitamin B_2 in the preparation of vitamin B_1 concentrates [Hogan and Hunter, 1928; Hogan and Richardson, 1932]. We, however, found partial destruction of both B-vitamins to occur when a yeast extract was exposed to ultra-violet radiation [Chick and Roscoe, 1929]. It is curious that during many years of vitamin B_2 investigation, we, in common with other workers, should have had no suspicion that this vitamin was sensitive to visible light. The materials used were subject to no special precaution, except that, as a routine, they remained in a dark refrigerator except when being handled. It is probable that, being chiefly yeast products of a brown colour, they were protected by this circumstance. Vitamin B_2 preparations from egg-white [Chick, Copping and Roscoe, 1930] are greenish yellow in colour and here sensitiveness of the vitamin to visible light was demonstrated in the following manner in some recent experiments.

A specimen (EW I) prepared in the ordinary way was found to be unusually poor in vitamin B_2 action and it was then realised that, owing to a change in arrangements, the preparation had been carried out in the month of July on a sunny bench under a window facing south-west. Ordinarily such preparations had been made in a north room and evaporated in a dark cupboard. Another preparation (EW II) was then made, in which the whole process, as far as possible, took place in the dark; this preparation proved to be unusually potent in vitamin B_2 action. The potency of EW I was much enhanced when a supplementary dose (20γ daily) of pure hepatoflavin was given; flavin, on the other hand, in no way enhanced the action of EW II. Addition of Supplement B (alkaline autoclaved yeast extract) increased the growth of rats receiving EW II, but did not assist the action of EW I. These facts indicated that it was the flavin constituent of the vitamin B_2 in EW I that had been destroyed by the sunlight, and that the growth-promoting power of this preparation, even when supplemented by Supplement B, was limited by the small amount of undestroyed flavin present.

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