

JUNE, 1936

RESEARCH BULLETIN 241

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

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Skin Lesions of the Rat Associated With the Vitamin B Complex

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(Publication Authorized June 3, 1936)



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TABLE OF CONTENTS

	Page
Abstract	4
Introduction	5
Experimental	5
Preparation of Water Extract of Yeast	6
Preparation of Flavin Concentrate	6
Preparation of Starch Extract	7
Preparation of Wheat Germ Oil	7
Method of Irradiation	7
Irradiation of Vitamin B Carriers in Solution and in Powder	7
Identity of Antidermatitis Factor	9
Differentiation of Two Types of Lesions	11
Observations on the Number of Factors in the Vitamin B Complex	13
Flavin and the Vitamin B Complex	16
Properties and Sources of the Antidermatitis Vitamin.....	18
The Effect of Visible Light on the Antidermatitis Vitamin	20
Unit for the Antidermatitis Vitamin	23
Discussion	23
Destruction of Antidermatitis Vitamin by Ultra-Violet Light	23
The Vitamin G Terminology	26
The Number of Factors in the Vitamin B Complex	29
Bibliography	35

ABSTRACT

If vitamin B carriers are irradiated as a powder it is estimated that 50-60 per cent of the antineuritic vitamin is destroyed, and 75-85 per cent of the antidermatitis vitamin is destroyed. If irradiated in solution less than 10 per cent of the antineuritic vitamin is destroyed, and over 90 per cent of the antidermatitis vitamin is destroyed.

The activity of the antidermatitis vitamin is only slightly reduced by intense exposure to the visible portion of the spectrum.

Evidence is presented that the antidermatitis agent is not one of the common unsaturated fatty acids, and that it is not vitamin E.

There are at least two types of skin lesions in rats due to a lack of vitamins. One is due to a deficiency of flavin, the other to a deficiency of the antidermatitis agent.

There are certainly three members of the vitamin B complex that are required by the rat: (1) the antineuritic vitamin, B in our terminology; (2) vitamin G which we regard as flavin, designated by us as the antidenuding factor; (3) the antidermatitis vitamin (provisionally designated as H). (4) A fourth, as yet unrecognized. The fourth is assumed because the three mentioned do not permit a normal growth rate, and do not permit the rats to attain normal mature weights. If there is more than one type of dermatitis it will be necessary to add a fifth.

Evidence is presented that flavin was the first limiting factor in the rations of the earlier workers, such as Smith and Hendrick, and Goldberger and Lillie.

Observations on the properties, and sources, of the antidermatitis vitamin are reported.

It is proposed that the unit for the antidermatitis vitamin be the daily dose required to heal dermatitis in 50 per cent or more of the rats, when the symptoms are definitely positive, but not unduly severe.

An attempt is made to correlate our observations with those of other workers.

Skin Lesions of the Rat Associated with the Vitamin B Complex

LUTHER R. RICHARDSON AND ALBERT G. HOGAN

It has been reported in previous papers (Hogan and Richardson '32, '34) that rats which receive irradiated vitamin B supplements develop a severe dermatitis and eventually succumb. The dermatitis is healed by tikitiki or by an alcohol extract of corn starch, but another type of skin lesion described as denuding, similar to that described by Goldberger and Lillie ('26), Sherman and Sandels ('31), also others to be mentioned later, eventually appears. This made it evident that our antidermatitis factor was distinct from vitamin G, as the term was then used. Before our first paper appeared Miss Reader had described lesions somewhat similar to those we had produced, which she ascribed to a deficiency of B₄. More recent papers from that laboratory state that the lesions can not longer be produced, and indicate that the vitamin B₄ hypothesis has been rejected. After our paper appeared several investigators, notably György and collaborators, described a dermatitis of rats, which is very similar to ours, if not identical with it.

We will return to these reports later for a more detailed discussion. More recently (Hogan and Richardson, '35, '36) additional evidence has been obtained which shows that except for a superficial resemblance the two types of lesions, denuding and dermatitis, have no relation to each other and are caused by two different deficiencies. The dermatitis is healed by an alcoholic extract of corn starch or by wheat germ oil, but is not healed by flavin. The denuded condition is healed by a flavin concentrate but not by wheat germ oil or an alcoholic extract of corn starch. The purpose of the present paper is to report other data on methods of differentiating these two deficiency diseases.

EXPERIMENTAL

The earlier papers ('32, '34) of this series should be consulted for a complete description of technical details. Albino rats were used as experimental animals, and the basal ration is No. 1669. This has been used satisfactorily for over 4 years and has the following composition:

Acid washed and alcohol extracted casein.....	20
Commercial sucrose	71
Salt mixture (Osborne and Mendel)	4
Cellulose	3
Cod liver oil	2

As a vitamin B carrier we have used an irradiated water extract of dried brewer's yeast.

Preparation of Water Extract of Yeast

The yeast extract was prepared as follows: 6 kilos of dried brewer's yeast were stirred with 15 gallons of boiling water, 75 cc. of glacial acetic acid were added, and the mixture was boiled for 3 to 5 minutes. After standing over night the clear liquid was siphoned off and concentrated to approximately 6 liters under reduced pressure and at a temperature not exceeding 60° C. An equal volume of 95 per cent ethyl alcohol was added and the precipitate was allowed to settle out. The supernatant liquid was drawn off and concentrated to a thick paste at a temperature not exceeding 50° C. If the temperature is too high during the later stages of concentration, the untreated concentrate will not sustain a normal growth rate, and if it is irradiated the animals will die before typical dermatitis has developed. Rats which receive 50 mg. daily (dry matter) of properly prepared untreated extract grow slowly until they weigh 100 to 125 gms., while those which receive 50 mg. of the irradiated extract invariably develop a severe dermatitis.

Preparation of Flavin Concentrate

The flavin concentrate was prepared by a modification of the method described by Kuhn, György, and Wagner-Jauregg ('33). The casein and albumin were precipitated from 50 gallons of skim milk, enough concentrated hydrochloric acid was added to make the solution deep purple to Congo red, and then it was treated with 200 grams of English fuller's earth. After stirring continuously for 4 hours the fuller's earth was allowed to settle until the following day when the supernatant liquid was siphoned off and discarded. The fuller's earth adsorbate was washed free from chlorides, covered with methyl alcohol, and stored in a dark bottle. Five such adsorbates were combined, the flavin was eluted with a mixture of 600 cc. of pyridine, 600 cc. of methyl alcohol and 2400 cc. of distilled water, and the pyridine-methyl alcohol eluate was concentrated under reduced pressure to a volume of about 200 cc. The suspended fuller's earth was coagulated by the addition of absolute methyl alcohol until a flocculent precipitate was formed, the precipitate was removed by centrifuging and the clear yellow-green fluorescent liquid was concentrated under reduced pressure to dryness. The residue was thoroughly extracted with 1500 cc. of 75 per cent ethyl alcohol. A flavin concentrate was then prepared from this alcohol solution by precipitation with barium hydroxide and silver nitrate as described by Stare ('35). This preparation retains considerable inert material and crystalline flavin was not isolated. In addition to flavin it contained some of the antineuritic vitamin but no others could be identified. In the comparisons made thus far our con-

centrate gave the same results as a commercial preparation,* and we have obtained no evidence as yet to suggest that it contains undesirable impurities.

Preparation of Starch Extract

Corn starch was extracted with hot 95 per cent ethyl alcohol, and the alcohol removed by distillation under reduced pressure. The residue was extracted with ethyl ether, and then the ether was removed by evaporation. The thick yellow oil that remained was approximately 0.3 per cent of the starch.

Preparation of Wheat Germ Oil

The wheat germ was extracted with U. S. P. ethyl ether, and the oil removed by evaporating off the solvent.

Method of Irradiation

Inasmuch as the technique has been changed the procedure now in use will be described in some detail. One hundred and twenty-five cc. of yeast extract containing 15 grams of dry matter are placed in a pyrex dish 28 centimeters long and 16.5 centimeters wide. The dish is enclosed in a metal jacket through which tap water flows constantly when in operation in order to prevent the temperature from rising. The jacket in turn is mounted on a mechanical rocker which keeps the solution continually agitated. The quartz-mercury arc is suspended at a distance of 14 centimeters above the solution, and the irradiation is continued for a period of 10 hours. Small quantities of water are added to the solution at intervals to replace the amount lost by evaporation. Needless to say the short exposure to ultraviolet rays employed for antirachitic activation does not bring about measurable destruction of the antidermatitis vitamin.

Irradiation of Vitamin B Carriers in Solution and in Powder

After we were assured that the yeast extract would give consistent results, the degrees of destruction of the antidermatitis vitamin when irradiated in solution was compared with the degree of destruction when irradiated as a dry powder. Likewise, the degree of destruction of vitamin B (B_1) in these same preparations was also determined. The degree of destruction of the antidermatitis vitamin was estimated by comparing the minimum amounts of the vitamin B carrier, when untreated and when irradiated, that are required to heal dermatitis. Twenty milligrams of the untreated yeast extract contained as much of the antidermatitis factor as 300 mg. of the same extract after it had been irradiated 10 hrs. in solution, and as much as 150 mg. when

*We are indebted to the Winthrop Chemical Co., Inc., New York City, for a generous supply of flavin.

it is irradiated as a powder, Tables 1-3. It is estimated, therefore, that about 85 per cent of the antidermatitis substance is destroyed when the powder is irradiated, and about 93 per cent is destroyed when the solution is irradiated.

TABLE 1.—COMPARATIVE DESTRUCTION OF THE ANTIDERMATITIS VITAMIN, WHEN IRRADIATED AS A DRY POWDER AND IN SOLUTION

Untreated			Irradiated		
No. of rats	Mgm. daily	Results	No. of rats	Mgm. daily	Results
Yeast extract (powder)					
3	30	all definitely improved	9	150	5 healed, 4 did not heal
5	20	4 definitely improved	5	100	4 did not heal
Yeast extract (solution)					
			3	300	2 healed, 1 did not heal
			5	200	1 healed, 4 did not heal
			5	150	did not heal
			5	100	did not heal

TABLE 2.—COMPARATIVE DESTRUCTION OF THE ANTINEURITIC VITAMIN, WHEN IRRADIATED AS A DRY POWDER AND IN SOLUTION

Untreated			Irradiated		
No. of birds	Mgm. single dose	Results	No. of birds	Mgm. single dose	Results
Yeast					
3	150	healed	5	300	healed
2	100	healed	3	200	2 healed; 1 improved but not healed.
3	75	healed slowly	-	---	-----
Yeast extract (powder)					
3	100	healed	2	200	healed
2	75	2 healed	2	150	1 improved slightly
2	50	not healed	2	100	not healed
Yeast extract (solution)					
			4	150	healed
			8	100	5 healed
			7	75	5 healed, 2 not healed

TABLE 3.—PERCENTAGE DESTRUCTION ON IRRADIATING VITAMIN CARRIERS IN DRY FORM AND IN SOLUTION

Supplement	Antineuritic vitamin ¹			Antidermatitis vitamin ²		
	Minimum to heal polyneuritis		Destroyed per cent	Minimum to heal dermatitis		Destroyed per cent
	Untreated mgm.	Irradiated mgm.		Untreated mgm.	Irradiated mgm.	
Yeast.....	75	200	62.5	50	200	75
Yeast extract powder.....	75	150	50.0	20	150	86
Yeast extract solution.....	75	75	10.0?	20	300	93

¹Pigeons as experimental animals.

²Rats as experimental animals.

The degree of destruction of the antineuritic vitamin was estimated by determining the minimum amount of the untreated, as compared

to minimum amounts of the irradiated, vitamin B carrier required to heal polyneuritis in pigeons. The birds were given ration No. 465 which consisted of commercial corn starch 58 parts, acid washed casein 20, cellophane 3, salts 4, cod liver oil 5, and lard 10. The substance to be tested for antineuritic activity was administered only to birds which had definite head retraction. If a pigeon had not improved within 24 hours after a dose of the test substance was administered, it was given a stock ration and allowed to recuperate before being brought down for a second test. On the other hand, if a bird showed some improvement 24 hours after receiving the supplement it was given a second dose and observed at the end of another 24 hour period. This was done in order that a temporary spontaneous improvement might not be construed as due to addition of vitamin B₁. It is observed from the data shown in Tables 2 and 3 that approximately 50 per cent of the antineuritic activity is destroyed when the vitamin B carrier is irradiated in powder, while less than 10 per cent is lost by irradiation in solution. Two grams of the powdered vitamin B carrier is all that can be irradiated at a single time as compared to 15 grams when irradiated in solution. Irradiation in solution, therefore, is the more satisfactory because there is less destruction of the antineuritic vitamin, greater destruction of the antidermatitis vitamin, and in addition a much larger quantity can be irradiated at a single time.

Identity of the Antidermatitis Factor

It has been shown (Hogan and Richardson '32) that if the ration contains corn starch the rats do not develop dermatitis. If the starch is extracted with strong alcohol the extract heals dermatitis, and if the ration contains extracted starch healing does not occur. When this alcohol extract of starch was treated with ether all of the active agent went into solution. It seemed probable that other vegetable oils might have antidermatitis activity, so a number were examined. It developed that wheat germ oil is very effective in healing the lesions while the other oils studied either did not contain the active agent or they are only slightly effective. The results are summarized in Table 4. Since cod liver oil, corn oil, flax seed oil, and walnut oil, are relatively ineffective in healing dermatitis, it seems improbable that the active agent is one of the unsaturated fatty acids, as might be suggested by the work of McAmis, Anderson, and Mendel ('29), of Burr and Burr ('30), and of Evans and Lepkovsky ('32).

More direct evidence that the antidermatitis agent is not a fatty acid was obtained by extracting yeast thoroughly with ethyl ether. The yeast was just as effective in healing dermatitis after extraction as it was before, and the ether extract was completely inactive. Like-

TABLE 4.—ACTIVITY OF OILS IN HEALING DERMATITIS

Source	No. of rats	Amount daily mgm.	Length of period days	Outcome
	2	50	12	declined
	2	70	9	recovered
Alcohol extract of starch.....	4	100	22	recovered
	2		45	declined
	3		20	recovered
	1	140	12	declined
Wheat germ oil, extracted with ethyl ether.....	2	25	5	declined
	2	50	20	recovered
	5		5	declined
	4	100	14	recovered
Wheat germ oil, cold pressed ¹	5	100	14	recovered
Corn oil (Mazola).....	1		26	recovered
	5	100	13	declined
Flaxseed oil, commercial.....	1		7	recovered
	3	100	32	declined
Flaxseed oil, cold pressed ¹	3		25	recovered
	1	100	21	declined
Flaxseed oil, extracted with ethyl ether.....	4	100	14	declined
Cocoonut oil ²	10	100	20	declined
	1		21	recovered
	3	500	16	declined
Walnut oil.....	1	100	14	recovered
	3		11	declined

1. Pharmaceutical Specialities Co., Chicago, Ill.

2. Durkee Famous Foods, Chicago, Ill.

wise, none of the active agent could be removed from yeast extract or from tikitiki by ether extraction. Although the antidermatitis substance is readily extracted from starch with hot alcohol, it can not be extracted with ether. However after the active agent has once been extracted with alcohol it then dissolves readily in ether.

Cod liver oil was included in the basal ration as a source of vitamins A and D, therefore the antidermatitis factor is neither of these.

The possibility that the antidermatitis agent is vitamin E has also been considered. Yeast is very effective in healing dermatitis, and the disease does not develop if the ration contains corn starch. Since the vitamin E free rations commonly used contain both yeast and corn starch, it seems impossible that the active agent in wheat germ oil could be vitamin E.

Although the antidermatitis agent is contained in some oils, we regard it as a water soluble vitamin because of its presence in a water extract of yeast and in tikitiki. Our tentative hypothesis assumes that the vitamin is coupled with some other constituents of the oil, though as yet we have been unable to effect a complete separation. Birch and György ('36) explain the antidermatitis activity of oils as a "sparing"

check the severity of the dermatitis or the decline in weight and all of the animals died (Graph VII, Fig. 1). If rats were given wheat germ oil, in addition to the basal ration No. 1669 and irradiated water extract of yeast, the dermatitis healed. However, after some initial gains the weights remained constant for a long time, then declined and finally the animals succumbed unless flavin was included with the other vitamin supplements (Graph V and VI, Fig. 1).

The next point to be considered is the effect of flavin and wheat germ oil as curative agents for the denuded condition. It was shown some time ago (Hogan and Richardson '34) that dermatitis is healed by tikitiki, but the animals made only slight gains in weight and in 12 to 16 weeks they develop a severe denuded condition similar to that described by Sherman and Sandels ('31). These symptoms had, at most, only a superficial resemblance to our type of dermatitis. It was also observed, as would be expected, that if the young rats received tikitiki as the sole source of the vitamin B complex from the beginning of the experimental period they became denuded in the same way in about the same length of time. The curative effect of wheat germ oil and of flavin was tested separately on denuded rats. One hundred mg. of wheat germ oil in addition to tikitiki did not have the slightest observable effect. The animals continued to decline and died in about the same length of time as the controls (Graph VIII, Fig. 1). Sixteen of the denuded animals were given 2 mg. dry matter of the flavin concentrate daily in addition to the tikitiki. These grew rapidly and the denuded areas were completely covered with a new coat of fur in 2 or 3 weeks. The combination of tikitiki and flavin, if given in large quantities, supplied a sufficient amount of the vitamin B complex to grow animals to maturity (Graphs I, II, III, and IV, Fig. 1 and Table 5). Preliminary reports of this work have been given elsewhere ('35, '36). It is demonstrated then that dermatitis is healed by wheat germ oil but not by flavin. On the other hand denuding is healed by flavin but not by wheat germ oil. Evidently the two types of lesions (see Plate I) are of entirely different origin.

A survey of the literature indicates that few workers are able to produce the denuded condition consistently, so we are including our method of producing these symptoms though in most respects it is identical with the procedure for producing dermatitis. The young rats are kept on the stock ration with their mothers until they are 21 days old. They are then placed in individual cages and given the experimental ration. If the rats are too small (weight of less than 25 to 30 grams) a large number will die before the denuded condition develops. It is our practice now, if some of the animals are too weak

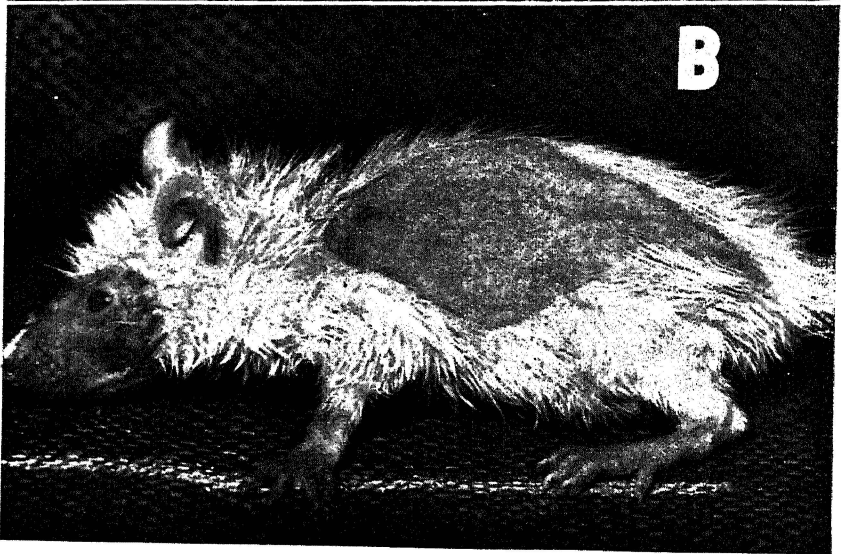


PLATE I

A. Received basal ration No. 1669 and irradiated yeast extract. Characteristic dermatitis on the feet, nose, ears, and mouth, but there was no loss of fur elsewhere. The dermatitis is healed by tikitiki, fuller's earth adsorbates, and wheat germ oil. It is not healed by flavin.

B. Received basal ration No. 1669 and tikitiki. Denuded areas on head, sides, and feet, but the encrusted lesions typical of the other type of dermatitis did not appear. The denuded condition is healed by flavin but is not healed by wheat germ oil.

to survive until they become denuded, to give them 10 cc. of fresh whole milk for 2 or 3 days. We use the same basal ration, No. 1669, and this is fortified with tikitiki (Wells, '21) as the sole source of the vitamin B complex. If this procedure is followed nearly all of the animals survive, and practically all of the survivors develop a typical denuded condition in 10 or 15 weeks. If the animals are kept on the stock ration until they are 28 days old, or if they weigh any considerable amount over 30 grams, there is a greater irregularity in the age at which the denuded condition develops. In some cases such animals were on the experimental ration 25 to 28 weeks before the symptoms appeared.

Observations on the Number of Factors in the Vitamin B Complex

A final decision as to the number of factors in the vitamin B complex must probably be deferred until each has been prepared in the pure state, but some progress can be made with the degree of isolation already attained. It is now possible to secure vitamin B (B_1) in crystalline form,* in a high degree of purity. The flavin concentrate described above is free from the antidermatitis agent. It possesses some antineuritic activity but contamination with known vitamins does not interfere with detection of those as yet unrecognized. Wheat germ oil is effective in healing dermatitis but completely inactive in healing polyneuritic pigeons, or in alleviating the denuded condition in rats. Separate sources of three of these vitamins are therefore now available, even though two of them are contaminated with others which have been previously recognized. It was decided therefore to test these three substances together as a source of the vitamin B complex. Such a test should yield additional evidence that the antidenuding and antidermatitis agents are not identical, and it might give an indication as to whether there exist still other previously unrecognized members of the vitamin B complex.

The procedure for making these tests was to produce both types of symptoms more or less simultaneous and study their response to various combinations of the three vitamins. Five rats with definite cases of dermatitis were each given 10 gamma of vitamin B_1 crystals and 4 mg. of flavin concentrate, in place of the irradiated yeast extract. The dermatitis became worse and all 5 died within 2 weeks without gaining in weight (Graph VI, Fig. 2). Three others with dermatitis were given 10 gamma of Vitamin B_1 crystals and 100 mg. of wheat germ oil in place of the irradiated supplements. The dermatitis was healed, but the animals made only slight gains in weight. The

*From the Merck Corporation, New York City.

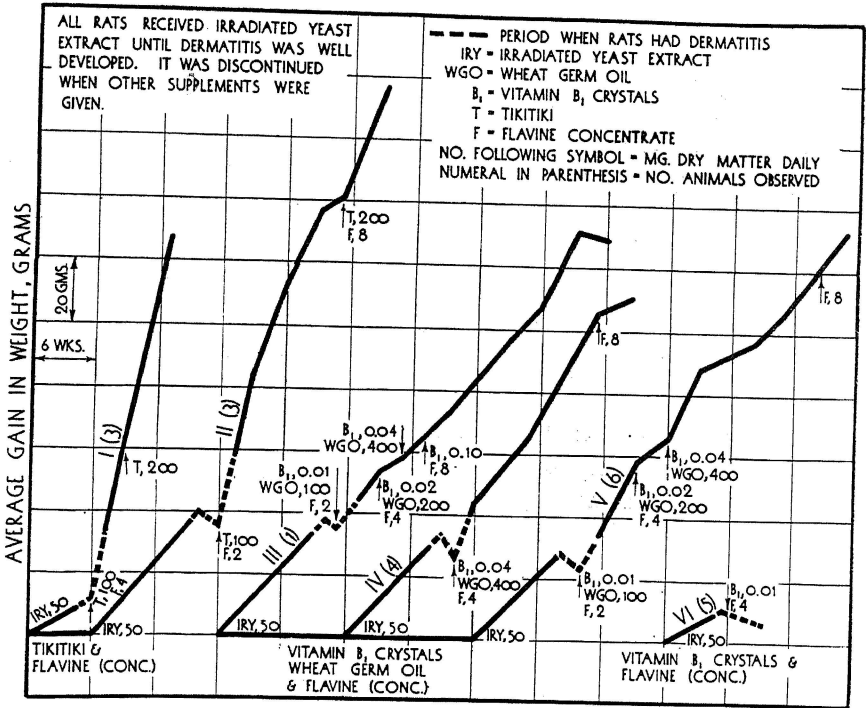


Fig. 2.—(a) Dermatitis is healed by tikitiki (I, II). 100 mg. tikitiki + 2 mg. of the flavin concentrate sustains a rate of growth that is only slightly subnormal.
 (b) The B₁ crystals + wheat germ oil + flavin healed dermatitis as promptly, and supported as rapid a growth rate as when the crystals were replaced by irradiated yeast extract. This combination seems incomplete, however, as normal mature weights were not attained, even though the amounts were greatly increased. Additional evidence that wheat germ oil contains an essential vitamin is shown (VI).

next step was to examine the effect of these substances on the denuded condition. Three rats which had become badly denuded on tikitiki were given 10 gamma of vitamin B₁ crystals and 2 mg. of the flavin concentrate, instead of tikitiki. The denuded condition healed promptly and the animals grew rapidly for 3 to 5 weeks, but the increase in weight was only temporary. Two rats which had started to denude were given 10 gamma of vitamin B₁ crystals and 100 mg. of wheat germ oil, in place of the tikitiki. The denuded condition became worse and the decline continued. When the condition of these animals became so serious that death was imminent 2 mg. of flavin concentrate were added to the other supplements. In 2 weeks they were normal in appearance and growing rapidly. These observations confirm those made with irradiated yeast as a source of vitamin B₁, and show that for our purpose the B₁ crystals may be substituted for the irradiated yeast.

The next step was to determine if all three supplements together are a complete source of the vitamin B complex. In a series of 18 rats

with well developed cases of dermatitis, the irradiated yeast extract was replaced with 10 gamma of vitamin B₁ crystals, 100 mg. of wheat germ oil, and 2 mg. of flavin concentrate. The dermatitis was healed in every case but though the rate of growth increased immediately the increase was only temporary. The supplement of 11 animals was increased to 40 gamma of vitamin B₁ crystals, 400 mg. wheat germ oil and 4 mg. of flavin concentrate, but there was no corresponding increase in the rate of gain (Graphs III, IV, and V, Fig. 2). The animals grew very slowly for a period of 20 to 25 weeks, but even then the two largest of the 6 rats that survived, 1 male and 1 female, each weighed only 173 grams. The other 5 died within 12 weeks. The flavin concentrate was eventually increased to 8 mg. daily, but this change did not have any effect on the rate of growth. It is believed 4 mg. was more than sufficient, since 2 mg. daily supported a fairly satisfactory rate of growth for several weeks when combined with tikitiki.

The observations just described would seem to show that the slow rate of growth cannot be explained by a deficiency of any one of the three vitamins under examination, and this point of view is sustained by other observations. For example increasing the vitamin B₁ crystals to 100 gamma did not materially accelerate the rate of growth (Graph III, Fig. 2).

Additional evidence that the vitamin B complex must contain at least 4 factors, is obtained by comparing the B₁ crystals with tikitiki as a source of the antineuritic vitamin. Tests on pigeons show that 15 gamma of the B₁ crystals contain as much of the antineuritic vitamin as does 100 mg. of tikitiki. Forty and 100 gamma of vitamin B₁ crystals contain 2.6 and 6.6 times as much antineuritic activity, respectively, as 100 mg. of tikitiki. However, rats which receive 100 mg. of tikitiki + flavin (Graphs I and II, Fig. 2) grow more rapidly and attain heavier weights than those that receive 100 gamma of the B₁ crystals + wheat germ oil + flavin (Graph III, Fig. 2). In other words a relatively small allowance of the antineuritic vitamin when supplied in tikitiki permits normal growth, while a larger allowance of B₁ crystals fails. Waterman and Ammerman ('35) reported that enormously larger doses of vitamin B₁ crystals are required for rapid growth than are required for a moderate rate. Under our experimental conditions this effect was not observed.

The data just cited show that the combination of vitamin B₁ crystals + wheat germ oil + flavin is not adequate for the normal growth of rats, and that it is inferior to tikitiki + flavin. Females on this latter combination may attain practically normal mature weights but

the males seldom exceed three-fourths of the average weight attained on a stock diet. This disparity may be largely due to differences in degrees of fat storage. The data are summarized in Table 5.

TABLE 5.—MAXIMUM WEIGHTS OF RATS ON SIMPLIFIED RATION AND VITAMIN SUPPLEMENTS; ALSO ON STOCK RATION

No. of rats	Sex	Supplement daily mgms.			Average maximum weight gms.
		tikitiki + flavin			
2	M	500	10		280
1	M	400	8		297
1	M	400	8		225
2	M	200	4		260
5	F	400	8		198
3	F	400	8		160
		B ₁ crystals + wheat germ oil + flavin			
1	M	0.10	400	10	173
1	M	0.04	400	8	153
1	F	0.04	400	8	173
2	F	0.04	400	8	134
5	F	0.04	400	8	93.6 all died
		Stock ration, ad libitum			
91	M				380
92	F				210

¹Picked at random from stock colony.

²Picked at random from stock colony before they were allowed to have litters.

In our opinion this superiority of tikitiki + flavin is very strong, though it may not be conclusive, evidence that there is a fourth factor required for normal growth. It is present in some degree in tikitiki, but is absent from the combination of vitamin B₁ crystals + wheat germ oil + flavin, or only present in subminimal amounts.

Flavin and the Vitamin B Complex

Our recognition of the fact that the old vitamin B is multiple in nature is based chiefly on the work of Smith and Hendrick ('26), and of Goldberger and Lillie ('26). Lack of space prevents a more complete review of the subsequent literature. The new vitamin designated as G in the United States and as B₂ in England. However, when it became evident that vitamin G itself was multiple there was considerable interest in determining which vitamin was the first limiting factor in the rations of the earlier workers. Our method of investigating this point was to produce in rats both types of the symptoms we have described, denuding and dermatitis, and then study the activity of the ration under examination as a curative agent.

It was evident from the first that the ration of Smith and Hendrick ('26) contains an abundance of the antidermatitis vitamin. When rats that had developed dermatitis by our procedure were transferred to the Smith and Hendrick ration the lesions healed and they made some gains in weight. When rats that had become denuded were likewise transferred to this ration some healed slowly, others did not heal at all, and in all cases the gains in weight were irregular. If the Smith and Hendrick ration was supplemented with flavin concentrate

the lesions were healed promptly and rats made considerable gains in weight. Other rats received a flavin concentrate that had been illuminated for 10 hours with a 1500 watt Mazda bulb, and they responded in the same way as rats that received no supplement at all. This is regarded as additional evidence that flavin was really the active agent in the concentrate employed. These observations are summarized in Table 6.

TABLE 6.—RESPONSE OF RATS, THAT ARE DENUDED OR HAVE DERMATITIS TO THE RATION OF SMITH AND HENDRICK

Supplement	Number of rats	Initial weight gms.	Final weight gms.	Gain gms.	Remarks
Denuded. Experimental period of 7 weeks					
2 mg. flavin conc. daily-----	3	68	164	96	healed
2 mg. illuminated flavin conc. daily-----	4	59	82	23	2 healed, 2 did not
none-----	4	78	101	23	2 healed, 2 did not
With dermatitis. Experimental period of 3 weeks					
none-----	4	52	74	22	healed

Inasmuch as Goldberger was the first to report "pellagra-like" skin symptoms in rats we have also included his ration in our observations. The procedure was the same as in studies of the Smith and Hendrick diet. The Goldberger ration also is very effective in healing dermatitis (Graph IV, Fig. 3). It does not contain the antidenuding factor (Graph V, Fig. 3), but if this ration is supplemented with our flavin concentrate it does heal the denuded condition, and supports some growth (Graph VI, Fig. 3). If the flavin concentrate is illuminated it no longer heals these symptoms, so we assume they are due to a lack of flavin, though one can not be certain until the pure compound is available. Similar experiments were carried out with Goldberger's black-tongue-producing ration ('28). This diet also healed dermatitis, but unlike the "rat-pellagra" ration it continued to support growth until the animals weighed 125 to 150 grams (Graph I, Fig. 3).

Four rats which were badly denuded were also given the black-tongue-producing ration. Two of them continued to decline and died in about 3 weeks (Graph III, Fig. 3). The other two improved, a new coat of short curly fur eventually covered the denuded areas, and they made some gains in weight (Graph II, Fig. 3). This shows that the black-tongue producing ration contains a suboptimal amount of the factor that prevents denuding, and may of course also be deficient in some other vitamin. Our interpretation of these results is they indicate clearly that the vitamin G of the earlier workers is identical with flavin.

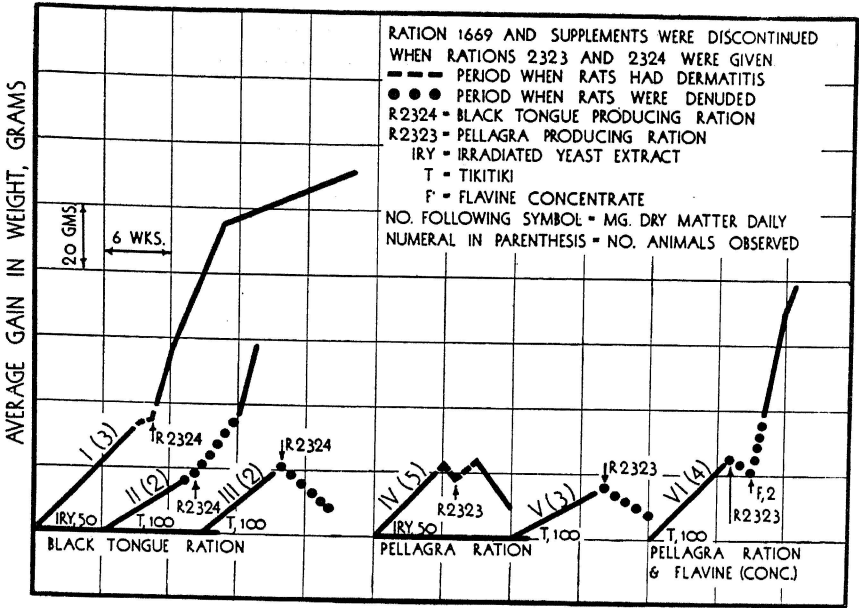


Fig. 3.—(a) Goldberger's "rat-pellagra" diet heals dermatitis, but is deficient in some other nutrient (IV). It does not heal denuding (V). The first limiting factor is flavin (VI). (b) Goldberger's black-tongue ration healed dermatitis (I). It contains suboptimal amounts of flavin (II, III).

Properties and Sources of the Antidermatitis Vitamin

After a method had been developed for producing experimental dermatitis consistently, a large number of substances that offered promise as vitamin concentrates were tested for antidermatitis activity. Four to 10 animals were used for testing a single material and the larger number has been used in most cases.

Some of the materials that were found to be active were fuller's earth adsorbates of yeast and of tikitiki, and alcoholic extracts of white corn. In addition to these, tikitiki and the alcoholic extract of corn starch have already been mentioned. Four fuller's earth (1, 2, 3) adsorbates* were tested. Fifty mg. daily of each of these preparations healed dermatitis, but there were only small increases in weight. After 3 to 6 weeks most of the animals became denuded, many almost completely, and all eventually succumbed. Although these adsorbates were prepared for use as vitamin B concentrates, 100 mg. were required to heal pigeons of polyneuritis. So far as can be judged by these methods then, both the antineuritic and antidermatitis vitamins had

* (1) Kindly supplied by Dr. R. R. Williams. (2) Kindly supplied by Fleischmann Laboratories. (3) Two were prepared in the laboratory by the method of Seidel, one from yeast, the other from tikitiki.

been concentrated to about the same extent. Recently it has been demonstrated that all of the antidermatitis vitamin can be removed from an aqueous solution of tikitiki by adsorption on fuller's earth. Seven hundred grams of dry matter of tikitiki were diluted to 2 liters with water. Twenty-five grams of English fuller's earth were added to the mixture and this was stirred with a mechanical stirrer for 3 hours and allowed to stand over night. The supernatant liquid was drawn off and treated twice with two separate 25 gram portions of fuller's earth. Fifty cc. of concentrated HCl were then added and the solution again treated with 25 grams of fuller's earth. The supernatant liquid was concentrated to its original volume and NaOH was added to neutralize the 50 cc. of acid. This fuller's earth preparation, as all the others, healed dermatitis. The solution of tikitiki which had been treated with the fuller's earth was completely inactive.

Another lot of tikitiki was made acid to thymol blue and treated 5 times with 5 separate portions of 25 grams of fuller's earth. The fuller's earth adsorbate healed dermatitis and the solution of tikitiki after treatment with fuller's earth also had some antidermatitis activity. In a strongly acid solution, therefore, the antidermatitis vitamin is only partially adsorbed, even by large amounts of fuller's earth. It was concluded that the active agent in the first preparation was all removed from the filtrate at the less acid reaction. This was confirmed by treating a third lot of tikitiki at a pH of 4.8 with 5 separate 25 gram portions of fuller's earth. The solution of tikitiki which had been treated with fuller's earth in this way was completely inactive in healing dermatitis. The adsorbate, like all other preparations, was active.

Birch and György ('36) found that vitamin B₆ is adsorbed by fuller's earth at pH 2.5 or 5.0, but a large amount of the fuller's earth was necessary in order to get anything like quantitative yield. The vitamin apparently was not adsorbed on acid clay. These results agree with ours.

Jansen's acid clay* was, unlike other adsorbates, more effective in healing polyneuritis than it was in healing dermatitis. One dose of 15 to 20 mg. usually relieved the characteristic symptoms of polyneuritis while 30 to 50 mg. daily for 10 to 20 days were required to heal dermatitis.

Vitamins B₁ and B₄ solutions, prepared by the Method of Kinnersley, O'Brien, Peters, and Reader ('33), are also concentrated preparations of the antidermatitis vitamin. Ten mg. of organic matter in each case healed dermatitis. Thus, the vitamin is adsorbed on charcoal in both neutral and acid solutions.

*We are greatly indebted to Dr. Jansen for his generosity in supplying this material.

Liebig's beef extract apparently contains a small amount of flavin, as the denuded condition was healed by a daily dose of 200 mg. Dermatitis was not healed by 300 mg. daily.

The reaction to heat and alkali has been proposed as a method of differentiating the various proposed members of the vitamin B complex. György ('35) has designated a factor as B₆ which is present in Peter's eluate, and which supplements vitamin B₁ crystals and lacto-flavin. It is not B₄ because it is stable to heat and alkali. According to Chick, Copping, and Edgar ('35) "Factor Y" or vitamin B₆, is the most stable to heat and alkali of any constituent of the B group of vitamins. It can resist prolonged autoclaving at a pH of 9.0.

Our antidermatitis vitamin is unstable to heat, for the antidermatitis activity of yeast is reduced by autoclaving at a neutral or slightly acid reaction. No effort was made to determine the precise degree of reduction but it is estimated that approximately 60 per cent was destroyed when the yeast was autoclaved 5 hours at a temperature of 120 degrees. Dermatitis was healed consistently with 60 mg. daily of untreated yeast, but between 150 and 200 mgs. daily of the autoclaved product were required for even occasional healing. Yeast that was autoclaved at an alkaline reaction by the method of Guerrant and Dutcher ('34) contained little or none of the curative agent.

These same preparations were also tested for their antidenuding activity. Denuding is healed by 100 mg. daily of yeast that was autoclaved at a slightly acid reaction. The material autoclaved by the method of Guerrant and Dutcher was inactive even at a level of 600 mg.

The vitamin dialyses through parchment paper. Dried yeast, the water extract of yeast, and tikitiki, do not lose any of their potency when thoroughly extracted with ethyl ether. An alcoholic extract of ether-extracted wheat germ contains the vitamin but is not as potent as tikitiki.

The Effect of Visible Light upon the Antidermatitis Vitamin

Kuhn, György, and Wagner-Jauregg ('33) were the first to demonstrate that flavin is destroyed by visible light and György ('35) has reported that his "antipellagra" factor, or B₆, is also destroyed when exposed to artificial light or when exposed for a long time to diffused day light. We reported in an earlier paper ('34) that a solution of equal parts by weight of tikitiki and liver extract does not lose its antidermatitis activity when exposed to a quartz-mercury arc through window glass. When wheat germ oil, tikitiki alone, and the water extract of yeast were included in our list of supplements, these experiments were repeated. 15 grams of the wheat germ oil were dissolved in 200 cc. of alcohol, and similar weights of dry matter of the other

preparations were made up to 200 cc. with water. The procedure for illumination was essentially the same as for ultraviolet irradiation. However, the tray was covered with plate glass one fourth inch thick. The solution was illuminated for 10 hours by a 1500 watt Mazda bulb, at a distance of 6 inches from the solution.

Rats which received the illuminated extract of yeast from the beginning of the experimental period never developed the well defined symptoms that are characteristic of dermatitis (Chart V, Fig. 4). Three of the 6 rats became denuded, and 2 died without becoming

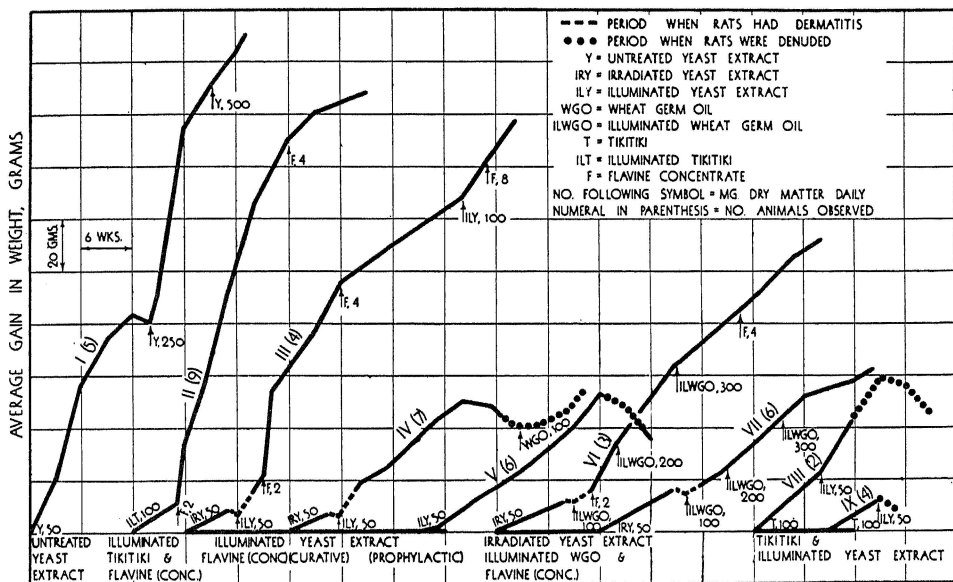


Fig. 4.—(a) Rats which received an illuminated water extract of yeast as the sole source of the vitamin B complex did not develop dermatitis (V). Five out of 6 became denuded and eventually succumbed. The denuded condition produced by supplying tikitiki as the sole source of vitamin B complex, was not alleviated by the addition of an illuminated water extract of yeast (VIII, IX).

(b) If illuminated wheat germ oil was added to the irradiated water extract (VI, VII), or if the irradiated water extract was replaced by an illuminated water extract of yeast (III, IV), dermatitis was healed. The gains in weight were slight unless they also received the flavin concentrate (III, VI).

(c) Rats which received illuminated tikitiki plus the flavin concentrate (II), as the sole source of the vitamin B complex did not develop dermatitis, and they grew as rapidly as when they received an untreated water extract of yeast (I).

denuded. The 6th rat developed symptoms that resembled dermatitis and was also denuded. The addition of 100 mg. of wheat germ oil daily did not heal the symptoms which were suggestive of dermatitis, though there may have been some temporary improvement. This condition, which may be called dual symptoms, has been observed occasionally in rats which received tikitiki, and is always healed by flavin. Illuminated yeast extract does not heal the denuded condition produced by tikitiki (Graphs VIII and IX, Fig. 4).

If rats which have developed dermatitis are given the illuminated water extract of yeast, the lesions are healed. However, after some initial gains in weight they again decline unless the illuminated vitamin B carrier is supplemented with flavin (Graphs III and IV, Fig. 4). Rats which receive the illuminated yeast extract, supplemented with flavin, grow just as rapidly or slightly better than those which receive equal amounts of the untreated yeast extract (Compare Graphs III and I, Fig. 4). Likewise rats on illuminated tikitiki supplemented with flavin (Graph II, Fig. 4) do just as well as those which receive equal amounts of untreated tikitiki supplemented with flavin. Evidently the antidermatitis agent was slightly or not at all affected by this procedure.

Illuminated wheat germ oil was also effective in healing dermatitis (Graphs VI and VII, Fig. 4), but there was evidence of some destruction. For example the combination of irradiated yeast extract + illuminated wheat germ oil + flavin did not support growth at as rapid a rate as when untreated wheat germ oil replaced the illuminated preparation. Our data on the degree of destruction are summarized in Table 7.

TABLE 7.—DESTRUCTION OF THE ANTIDERMATITIS VITAMIN IN WHEAT GERM OIL BY ILLUMINATION

No. of rats	Illuminated wheat germ oil mgm.	Healed	Not healed
a 10	100	7	3
b 18	100	6	12
11	200	11	0

György ('35) has noted that all of the solution containing B₆ are colored yellow and under the influence of diffused light these solutions take on a darker, reddish brown color. We have not been able to detect any change in the color of our solutions of yeast extract and tikitiki. They are a dark, reddish-brown color, before and after illumination. The wheat germ oil, however, which has a decided yellow color becomes somewhat lighter on illumination. It may be that differences in depth of color may explain why we observed less destruction than did György.

While it is possible that our flavin concentrates contain vitamins other than flavin, it is certain that they do not contain the antidermatitis agent. In fact the original whey concentrate, from which some of our flavin concentrates were made, did not have any antidermatitis activity when fed at a 500 mg. level. Since neither the flavin concentrate nor the original whey concentrates prevented or cured dermatitis, the only other source of this vitamin was the illuminated yeast extract or illuminated tikitiki. We believe these data, together with the fact that all illuminated supplements are effective in healing dermatitis, are

conclusive evidence that under our conditions there is little destruction of the antidermatitis agent by illumination. Tests made on pigeons with polyneuritis indicate that illumination does not destroy a measurable amount of the antineuritic vitamin.

Unit for the Antidermatitis Vitamin

As a unit for the antidermatitis vitamin we suggest the daily dose required to heal dermatitis in 50 per cent or more of the animals, when the symptoms are definitely positive but not too severe. It has been our experience that even large doses will not always heal the disease if it is too far advanced. We prefer to use the amounts that heal dermatitis as a unit, rather than the amount that sustains a definite growth rate, for the reason that substances such as tikitiki and wheat germ oil are effective in healing dermatitis, but they do not support growth because of the deficiency of flavin. In our experience the curative method gives sharper distinctions and requires less time than the prophylactic, though undoubtedly either can be used.

DISCUSSION

Destruction of Antidermatitis Vitamin by Ultra-Violet Light

Several workers have stated that they were unable to confirm our earlier reports in some respects. It is obvious now that these failures were at least partly due to the fact that the technique in use at that time was inadequate for the differentiation of members of the vitamin B complex. The first paper of this series (Hogan and Hunter '28) was originally designed to show that the vitamin B is multiple in nature. It was realized that the destruction of any newly recognized members of the complex was probably incomplete, and that there may have been some destruction of the antineuritic vitamin. For that reason Hogan and Hunter did not suggest that the irradiation method could be used for a quantitative measurement of the new factor. When they later attempted to devise a quantitative procedure difficulties were encountered which showed that the technique was inadequate for that purpose. The degree of success attained subsequently can be estimated from the papers previously reviewed. These show that the inconsistencies were largely due to the variations in the amounts of the antidermatitis factor contributed by the starch and milk fat in the basal ration. Even by our present technique some 10 per cent of the antidermatitis agent escapes destruction, but we do not regard that as a serious weakness. The biological method at best is not highly accurate, and for the present the method of irradiation offers certain definite advantages. It is be-

lieved that this technique can be usefully applied until each of the essential vitamins is available in a highly purified form.

Shortly after the paper of Hogan and Hunter appeared reports on their technique were published by other laboratories. Kennedy and Palmer ('29) did not detect appreciable destruction of any of the vitamins of yeast. The knowledge now available indicates that several factors may have contributed to their inability to repeat our observations. In the first place, their basal ration may have contained more of the factor which was supposed to be destroyed by the irradiation of yeast than ours had. Their experimental diet contained 9 per cent of butter fat and 67.3 per cent of tapioca dextrin. In our experience this factor is always present in milk fat. It is also present in corn and rice starch, so it may have been present in the tapioca dextrin.

A second explanation for the discrepancy is the difference in the method of irradiation. We spread the yeast out in the thinnest possible layer, never more than 0.5 mm. thick, and this was frequently stirred. Kennedy and Palmer irradiated a layer 2 mm. thick, which presumably reduced the intensity of the irradiation by one fourth.

The third explanation is found in the different level at which the yeast was supplied. Kennedy and Palmer began at a level of 500 mgm. daily per rat. We began at a level of 200 mg. daily and then slowly increased this allotment whenever necessary in order to keep all the rats that received the mixture of irradiated and autoclaved yeast in a reasonably healthy condition. Our object in this first paper was to show that the old vitamin B was a mixture, and this could not be done unless the yeast preparations were supplied at a minimum level.

Kennedy and Palmer reported much less destruction of the new factor than we had observed, but the next paper on this topic to appear, by Chick and Roscoe ('29), seemed to report considerably more. They confirmed our observation that the vitamin they designated as B₂ was destroyed, but in their hands the destruction of B₁ was much more complete than our data had indicated. In the light of more recent advances in our knowledge of the vitamin B complex, we believe some of the discrepancies between the report of Chick and Roscoe, and our later papers, find ready explanation.

Chick and Roscoe ('29) irradiated their vitamin B carrier in solution, as we do also at present, but they observed less destruction of their "B₂," than we have reported (see Table 3). Our explanation of this discrepancy is their basal ration contained an appreciable amount of the substance that they were attempting to destroy by irradiation. Their basal ration contained 60 per cent of rice starch, and our observations have shown that this material contains the anti-dermatitis vitamin.

As was just mentioned these workers reported a greater degree of destruction of B_1 than we have been able to detect. Before considering any possible reasons for our failure to agree completely with them on this point it is necessary to review their experimental procedure. Chick and Roscoe apparently used two basal diets. In one of these they included egg white, both as a source of protein and of vitamin B_2 . In the other diet the egg white was replaced by casein, and B_2 was supplied in a separate supplement of 0.4 gm. daily of autoclaved yeast. They do not state which diet was used when they were studying the effects of ultraviolet light. Vitamin B_1 was supplied as an extract of yeast, both irradiated and non-irradiated. The degree of destruction in the irradiated material was estimated by comparing the growth rate it supported, with that obtained on the untreated preparation. It may be of course that the degree of destruction indicated by their method is correct, but we have considered other possibilities. Admittedly any attempt to explain this discrepancy must be largely speculative, because of the confusion that now surrounds the vitamin B complex, but the following possibilities have been considered.

(1) The decreased growth rate may have been due to the destruction of some vitamin other than B_1 . Their method assumes that either the 0.4 gm. of autoclaved yeast or the egg white, contains an adequate amount of all members of the complex except B_1 . Recent work indicates that this assumption is unjustified. Our own observations show that autoclaving partially destroys both flavin and our antidermatitis vitamin. If egg white were the source of B_2 normal growth could hardly be expected when the yeast extract was irradiated. It is now well known that a combination of the antineuritic vitamin and flavin is not adequate as a source of the vitamin B complex. This view is supported by their protocols. There is only one case in which the rate of growth on different levels of the irradiated extract can be compared. Two rats grew at an average rate of 7 grams per week on 0.12 gm. daily, and 1 rat grew at an average rate of 8 grams per week on 0.25 gm. daily. If vitamin B_1 had been partly destroyed, doubling the dose should surely increase markedly the rate of growth. If, on the other hand, some vitamin had been more or less completely destroyed, doubling the dose would have little effect on the growth rate.

(2) Different methods were used in testing for destruction of the antineuritic vitamin. Our estimate was based on the amounts necessary to heal pigeons of polyneuritis, theirs was based on differences in the growth rate of rats. We have never had any reason to doubt that the curative test on pigeons is specific for the antineuritic vitamin, but we do regard the rat-growth method as unreliable, at least unless

advantage is taken of the more recent improvement in technique. If vitamin B₁ crystals are used as a control for the test substances we would expect the rat-growth method to yield dependable results.

Fundamentally this second suggestion is the same as the first, for it rests on the assumption that the retarded growth rate described by Chick and Roscoe was not due solely to a deficiency of vitamin B₁.

Thatcher, Sure, and Walker ('30) and Sure, Smith, and Kik ('31) were able to make effective use of the irradiation technique, though they did not estimate the degree of destruction of any of the members of the vitamin B complex.

György ('35) has reported that he confirmed our work in every essential respect, but he made one reservation: Some of his animals required a long "preparatory period" before the symptoms developed, and others healed spontaneously. It is our opinion that this discrepancy is at least largely due to the makeup of his experimental ration, which contained 68 per cent of rice starch and 9 per cent of milk fat. We have never been able to produce dermatitis if the experimental ration contains any considerable amount of corn starch. We attempted sometime ago to substitute rice starch for the sucrose in Ration 1669, but it was quite evident that rice starch is almost if not quite as objectionable as corn starch. The rice starch was supplied to 6 rats with dermatitis. Three definitely healed and the others made some improvement. It is also our experience that milk fat interferes with the development of these lesions. If György's B₆ is the same as our anti-dermatitis vitamin the use of rice starch and butter fat in his ration could explain the numerous spontaneous healings he observed. We have not observed a single case of spontaneous healing of dermatitis by the use of our present technique and these observations include 550 cases of dermatitis.

As to the length of the preparatory period, rats subjected to our technique ('32, '34) develop dermatitis in 5 to 6 weeks on the average, when they are between 8 and 9 weeks of age. György ('35) reports an average time of 7 weeks. If our experimental procedure is followed every rat that survives the depletion period will develop dermatitis, and every one will succumb unless remedial measures are applied.

The Vitamin G Terminology

After flavin was accepted as a member of the vitamin B complex, considerable discussion developed concerning the position it should occupy in the vitamin alphabet. In deciding what terminology should be eventually adopted it should be remembered that in the past at least two different deficiency diseases have been associated with vitamin G (B₂). In our view this means that no terminology based on

the old nomenclature will be entirely logical and we can only choose which compromise should be adopted.

The first clear cut lesions associated with a deficiency of vitamin G in the past, were described by Goldberger and Lillie ('26). Rats were used in these studies and the experimental ration was made up as follows: Purified casein 20, salt mixture 4, hydrogenated cottonseed oil (Crisco) 3, cod liver oil 2, corn starch to make 100. The antineuritic vitamin was supplied by percolating corn meal with 85 per cent alcohol, and drying the extract on corn starch, which was then included in the ration.

If historical values are to be considered in the adoption of a terminology it is necessary to know what type of lesions Goldberger produced. They are described as follows:

"After a variable period following the arrest of growth already mentioned, there has been observed in many of the animals so fed a tendency for the lids of one or both eyes to adhere together with, in some instances, an accumulation of dried secretion on the margin of the lids. At about the time or shortly after the appearance of this ophthalmia there has developed in nearly, if not quite, every one of the animals on the indicated diets, some loss of fur. This fur loss has in some begun in irregularly distributed patches. More commonly it has been observed to begin at the side or over the top of the head, the sides or front of the neck, or in the region of the shoulders. From these initial sites the depilation has extended and in some of the animals had led to almost complete denudation of the head, neck, and trunk. The initially affected sites and, in the early stages, the areas involved by the spreading depilation have, in many of the animals, been sharply delimited and bilaterally symmetrical.

"With or without such loss of fur some of the animals have developed a dermatitis at one or more of the following sites: Ears, front of neck and upper part of chest, forearms, backs of forepaws, shins, and the backs of the hind paws. This dermatitis, particularly as it has affected the paws, forearms, neck, and ears, has been sharply outlined and bilaterally symmetrical. To the eye it has differed somewhat with the site affected. The ears seemed definitely reddened and thickened with what appeared to be a yellowish incrustation of dried serum. In healing, desquamation took place, leaving the skin of the pinna with a polished, glistening, somewhat parchment like appearance. In one animal in which the dermatitis involved an extensive butterfly shaped area on the front of the neck and upper part of the chest, the affected skin was red and, at first, apparently superficially eroded and moist, then, like the ears, became dry, incrustated and rough. In the cases in which the backs of the forepaws were affected, the skin was red and rough and, after healing, but before the renewal of the normal fine, silky fur, the skin had a pale pink, glistening, new-skin appearance. The backs of the hind paws, when affected, presented at first an appearance as of a matting of the silky fur of this part, which then looked dull and thickened. Later this matted layer of fur began to fissure and to crack and then gradually desquamated, leaving a denuded pale pink, glistening skin. The shortest period so far recorded within which this dermatitis has appeared has been approximately seven weeks. In a few of the cases so far observed, the affected animals have presented a linear fissuring or ulceration at the angles of the mouth. In a somewhat larger number there has occurred a lesion at the tip of the tongue, which first appeared as a small, roughly circular, grayish opacity or bleb, or as an ulceration which, in some, went on to the formation of a localized yellowish slough. In one of such animals there was evidence also of an inflammation of the anterior part of the floor of the mouth. In two, diarrhea was present."

It is commonly assumed that the lesions described by Goldberger and Lillie were not due to a deficiency of flavin, but of one of the more recently described antidermatitis agents. For example György ('35) states that lactoflavin has no causal relationship to the rat dermatitis first produced by Goldberger and Lillie. Some of the observations that lead us to a different interpretation were summarized in Fig. 3. Other reasons are as follows. So far as one may judge by the description the lesions reported by Goldberger and Lillie are identical with those observed by Sherman and Sandels ('31), by Bing and Mendel ('29) and by ourselves in our studies of flavin deficiency. This conclusion is supported by examination of the experimental procedures. There were no obviously important differences in the basal rations. Goldberger used an alcohol extract of white corn as source of vitamin B, Sherman and Sandels used an alcohol extract of wheat, Bing and Mendel, as did we, used tikitiki. The symptoms reported by Goldberger and Lillie are frequently described as dermatitis, and that designation may be correct, but with minor exceptions the symptoms are entirely distinct from the dermatitis we have been studying. The lesions we describe as denuding are healed promptly and decisively by our flavin concentrate and we regard it as almost certain that flavin would have healed the lesions reported by the other three groups of workers. Whatever this substance may be there is ample precedent for designating it as vitamin G. Some of the confusion now surrounding the vitamin B complex will be dissipated when it is recognized that the lesions reported by the earlier workers were essentially identical and that all were due in some degree at least to a deficiency of flavin.

The next report of definite lesions associated with deficiency of a vitamin was made by us about 4 years ago ('32). If our present system of nomenclature is retained this factor becomes vitamin H.

We will add that in our experience the symptoms usually agree with those described in the first paragraph of the excerpt from the paper by Goldberger and Lillie. The more severe symptoms described in the second paragraph are much less common. These cases are very similar to those we have designated as dermatitis, except for the loss of fur, and except for that difference would be indistinguishable. It is possible that this condition is due to a multiple deficiency, but since it is healed by our flavin concentrate we have for the time being rejected that hypothesis.

Two years after Goldberger described this "pellagra-like" disease in rats he reported the production of experimental black-tongue in dogs, but the evidence now available does not enable one to decide whether Goldberger's "rat-pellagra" and black-tongue are analogous.

The black-tongue-producing ration contains some of the factor that prevents and heals denuding, but when used as a diet for rats the amount is certainly below the optimum. It is certain, though, that our antidermatitis vitamin is not the first limiting factor in Goldberger's pellagra- or black-tongue-producing rations, for they rapidly heal dermatitis. However, if rats which have become denuded are given Goldberger's ('26) pellagra-producing ration this condition is not healed unless they are also given flavin. The black-tongue ration healed the denuded condition in some cases, therefore it is concluded that it contains a small, but for the rat inadequate, amount of flavin.

Other workers have investigated black-tongue- and pellagra-producing rations in more detail but some of their conclusions are not in agreement. According to Birch, György, and Harris ('35) the anti-black-tongue factor is neither vitamin B₆ nor lactoflavin. They also asserted that the rat is not susceptible either to pellagra or black-tongue. Booher and Hansmann ('36) reported that black-tongue is healed by a flavin concentrate. However, this preparation contains at least one other, as yet unrecognized, member of the G complex. Koehn and Elvehjem ('36) reported that flavin is ineffective in healing black-tongue but the disease is healed by a flavin-free concentrate prepared from liver. Dann ('36) stated that human pellagra is not healed either by flavin or by vitamin B₆. The publications clearly indicate that one or two additional members must be added to the vitamin B complex, depending on whether human pellagra and canine black-tongue are identical.

The Number of Factors in the Vitamin B Complex

The data we have presented in this paper show conclusively that there are at least three vitamins in the vitamin B complex, and that a deficiency of any one causes a specific disease. Convenient sources of the three vitamins are vitamin B₁ crystals, wheat germ oil, and a flavin concentrate. There is ample justification for assuming the existence of a fourth, however, for the reason that rats which receive the three mentioned do not attain normal mature weights. For example rats that receive B₁ crystals, flavin, and wheat germ oil do not attain normal weights (Table 5), though those on tikitiki and flavin do. There may be some explanation, but according to our working hypothesis tikitiki, and some other supplements, contain a fourth water soluble factor that is required by the rat for normal development. It is entirely possible of course that such a factor would turn out to be plural in nature.

The question arises, though, is the antidermatitis vitamin identical with any of the other factors which have recently been proposed as components of the vitamin B complex.

Chick and Copping ('30) demonstrated that alkaline autoclaved yeast or alkaline autoclaved yeast extracts, supplement a ration which contains Peter's concentrate as the source of vitamin B (B_1) and egg-white as the sole source of vitamin G (B_2). They concluded that there was a factor "Y" in the autoclaved supplements which was not present in any other component of the ration. Chick, Copping, and Edgar ('35) recognized that there are two distinct types of skin lesions in rats caused by deficiencies of members of the B complex. According to our interpretation of their paper the "general" type of lesion was associated with a deficiency of flavin, and the florid type was associated with a deficiency of their Y factor. Consistent healing of either type was not obtained, however, unless both supplements were supplied simultaneously. This is not in accord with our experience.

In 1935, György ('35) showed that rats which receive vitamin B_1 crystals and crystalline flavin develop a "rat pellagra." If they receive in addition to vitamin B_1 and flavin, Peter's eluate, or a solution which had had the flavin removed by adsorption on fuller's earth, "rat pellagra" does not develop and the animals grow until they attain weights of 150 to 170 grams. When the rats are given vitamin B_1 and Peter's eluate without flavin "rat pellagra" does not develop but another more generalized type of skin lesion, in which there was a loss of hair develops in some of the animals. This latter condition is healed by lactoflavin. We assume that this latter condition is similar to the denuded condition in our rats, which is also healed by flavin. It would seem that the "rat pellagra" which is healed by Peter's eluate, vitamin B_6 , is the same as our dermatitis if it were not for two conditions. Namely, vitamin B_6 is destroyed by visible light and is alkali-heat stable. The vitamin which prevents our dermatitis is only slightly affected by visible light, and it is destroyed by heat and alkali. When it becomes possible to use a more refined technique these discrepancies may disappear.

Stare ('35) used a basal ration of casein, sucrose, Crisco, cod liver oil, and salts, supplemented with a highly concentrated vitamin B_1 preparation. The rats did not develop dermatitis consistently, and on the basis of our experience we would explain the variability by the presence of Crisco (hardened cottonseed oil) in the diet. The lesions were not prevented by flavin, but they were prevented by liver extract from which the flavin had been removed.

Our experience is at variance with that of Stare, in that the filtrates we obtain after treatment with fuller's earth do not heal dermatitis. We also infer that Stare did not observe specific symptoms due to a deficiency of flavin.

Halliday and Evans ('36) used the basal ration of Sherman and Spohn ('23) which contains 68 per cent of corn starch and 8 per cent of butter fat. The diet was modified to the extent that the casein was extracted with hot concentrated alcohol as well as with cold 60 per cent alcohol. Rats that received this basal diet, supplemented with a vitamin B₁ concentrate, developed dermatitis. Alopecia was not observed. Since the ration of Halliday and Evans contained corn starch our data indicate that rats which received it would develop dermatitis rarely or not at all. As a matter of fact, though, they apparently produced lesions more consistently than Stare did.

The observations of these workers on the properties of their antidermatitis agent are in agreement with those of Stare. A fuller's earth adsorbate of yeast or liver extracts did not heal the lesions, which indicates they were not due to a lack of flavin. The filtrates from the fuller's earth prevented the lesions. In addition they report that the effective agent was not destroyed by autoclaving for one hour at a pH of 9.

Our experience is at variance with that of Halliday and Evans in some respects. (1) We have been unable to produce dermatitis if the ration contained corn starch and milk fat. (2) Every fuller's earth adsorbate of yeast extract that has been tested heals dermatitis. (3) The filtrate is devoid of activity, in other words the adsorption of the antidermatitis agent is complete. (4) Our factor is destroyed by heating in an alkaline medium. However we heated the vitamin carrier for five hours instead of one.

The brief abstract of Booher's ('36) observations indicates that the factor she is investigating is identical with our antidermatitis vitamin. An active concentrate can be prepared from rice polishings; it is relatively stable in an acid or neutral medium; it is readily destroyed by alkali; in its absence rats fail to grow and develop skin lesions. We would add that they soon succumb unless rescued by a curative agent.

Reader ('29, '30a) presented what seemed at the time to be convincing evidence for the existence of a third member of the B complex. In her earlier papers this evidence was based on the rate and extent of growth of rats. In a later paper (30b) dermatitis was produced in adult rats, and the vitamin B₄ was assayed by a curative test. The symptoms that are important for our purpose were swollen red paws. The basal diet was made up of casein 20, rice starch 70, agar 2, salts 5, cod liver oil 3. Vitamin B₁ was supplied as a concentrate, B₂ as a yeast extract heated at 120° for one hour at a pH of 9. It seemed

at the time that Reader had made a very significant contribution, but more recent papers from that laboratory, (O'Brien, '34, and Kinnersley, O'Brien, and Peters '35) would repudiate the earlier claims. These later reports state that the original vitamin B₁ concentrates failed either to cure the specific symptoms or to support increases in weight, but in later attempts such concentrates could not be prepared. In addition the condition of pink feet could no longer be produced. These later workers apparently believe that all the symptoms described by Miss Reader were due to a sub-acute deficiency of vitamin B₁.

Inasmuch as our knowledge of none of these vitamins is complete, and in many respects is only fragmentary, it is impossible to identify the lesions described by Miss Reader with those produced by other workers. Since red swollen paws are seldom or never identified with a deficiency of vitamin B₁ alone, we believe the interpretation of Kinnersley, O'Brien, and Peters ('33) and of O'Brien ('34) should be accepted with caution. It seems to us that the dermatitis described by Reader may be identical with, or similar to, that produced by other of the more recent workers. Until the properties of these antidermatitis agents are more clearly defined a high degree of variability may be expected, so we believe the later failures may be explained by contamination of the basal diet with the vitamin under investigation. This would explain why in their more recent work Kinnersley et al. ('35) were unable to produce the characteristic lesions and why they were unable to prepare vitamin B₁ concentrates with properties similar to those first used. Our antidermatitis agent is present in some degree in rice starch, which they used, and in our hands autoclaved yeast is not a dependable source of any vitamin. In our view the B₄ of Miss Reader is probably not identical with flavin; it may be identical with our antidermatitis agent, or with that of György ('35), Chick, Coping, and Edgar ('35), of Stare ('35), or of Halliday and Evans ('36).

The observations of Bender, Ansbacher, Flanigan, and Supplee ('36) agree in almost every detail with our earlier reports. The substitution of sucrose for dextrin was followed by a high incidence of dermatitis, and when the amount of hydrogenated cottonseed was reduced from 10 to 3 per cent every animal developed lesions. The disease was not healed or prevented by lactoflavin, but a concentrate prepared from rice polishings was highly active. These investigators used crystalline vitamin B₁ and crystalline lactoflavin as supplements to the basal diet. These investigators made no comment on the chemical properties of the active agent, or on its stability, but the photograph reproduced in their paper indicates that the lesions are identical with those we have observed.

Elvehjem and Koehn ('35) and Lepkovsky and Jukes ('35) produced a dermatitis in chicks which is not healed by flavin. Stare ('35) described similar lesions in chicks and he also reported that flavin is not the curative agent. It is uncertain whether the anti "chick-pellagra" factor is identical with György's B₆, but if the lesions are not healed by wheat germ oil we assume they could not be due to a lack of our antidermatitis vitamin.

In order that the discrepancies may appear more clearly some of the more important ones have been summarized in Table 8. (Page 34).

One is reluctant to conclude that symptoms which are apparently identical could be of diverse origin, but we are not disposed to deny the possibility unless the discrepancies can be satisfactorily explained. It should be remembered though that there are undoubtedly minor differences in the constituents of the various rations used, and some are less contaminated than others with the vitamins under investigation. The vitamin carriers themselves do not have the same degree of purity. Other variations in procedure cause the symptoms to appear at unlike ages, and the symptoms due to acute and chronic deficiencies may not be precisely the same.

On the basis of our own experience then, we list the members of the vitamin B complex required by the rat as follows:

Antineuritic vitamin	B
Antidenuding vitamin (flavin)	G
Antidermatitis vitamin	H ?
Unknown	at least one

If future work does not resolve the discrepancies cited a second antidermatitis vitamin must be included.

It may be added that unnecessary confusion will be avoided if the vitamin requirements of various animals are classified separately. Our experience with pigeons (unpublished) has convinced us that their requirements are quite different from those of rats. The knowledge now available does not indicate definitely that they have a common requirement for any member of the vitamin B complex except the antineuritic vitamin, though future studies may disclose other common requirements. Various pathological states of the chick have been ascribed to specific vitamin deficiencies, but these studies have not proceeded far enough to show whether or not the factors concerned are also required by the rat.

TABLE 8.—COMPARISON OF PROPERTIES ASCRIBED TO ANTIDERMATITIS VITAMINS

Reported by	Designation	Effect of heat in alkaline medium	Adsorption	Effect of light	Symptoms
Gyorgy ('34)-----	B ₆	stable	adsorbed on fuller's earth quantitatively at pH 2.5 or 5.0	destroyed by visible light	specific acrodynia-like dermatitis
Chick, Copping and Edgar ('35)-----	Y or B ₆	stable	-----	-----	?
Stare ('35)-----	B ₂	-----	not adsorbed on fuller's earth	-----	dermatitis
Halliday and Evans ('36)	B ₆ ?	stable	not adsorbed on fuller's earth	-----	acrodynia-like dermatitis
Reader ('30)-----	B ₄	unstable	adsorbed on charcoal at pH 1	-----	muscular weakness red swollen paws
Hogan and Richardson	antidermatitis or vitamin H	unstable	adsorbed on fuller's earth quantitatively at pH 4.8	destroyed by ultraviolet, not destroyed by visible	characteristic dermatitis
Booher ('36)-----	H	unstable	-----	-----	abnormal skin

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