CXXIV. THE WATER-SOLUBLE B-VITAMINS.

VI. FLAVIN AND VITAMIN B₆ IN CEREALS.

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IN the course of investigations on the skin symptoms in rats due to lack of vitamin B₂ and of its separate components, it was found advisable to study the effect of the Bourquin and Sherman [1931] type of diet. This diet has been widely used in the study of vitamin B₂, and recently by Kuhn *et al.* [1933, 1]. It was originally devised to be free from vitamin B₂ and to contain vitamin B₁ as an alcoholic extract of whole wheat.

Booher [1933; 1934] demonstrated that when this diet was supplemented with flavin good growth was obtained, but Kuhn *et al.* [1933, 2] and György *et al.* [1934] found that some further factor obtained from yeast, and considered at that time to be vitamin B₄ [Reader, 1929], was required as well as flavin to supplement the diet completely. Nevertheless, flavin was assumed to be identical with vitamin B₂. Later results published by Euler *et al.* [1934] and György [1934; 1935] and also the observations of Chick *et al.* [1935] showed that vitamin B₂ was composite in nature, that flavin was only one component, and that a supplementary substance was also present, which was named vitamin B₆ by György.

In this laboratory preliminary tests with Bourquin and Sherman diets confirmed the observations that the growth of rats could be stimulated when this diet was supplemented with flavin and indicated that while flavin was lacking in the alcoholic cereal extract present in the diet, vitamin B₆ was present in abundance. This was confirmed by Birch and György [1935] and this conclusion is also supported by observations reported in another paper [Copping, 1935; 1936] on the relation between the constituents of vitamin B₂ and the occurrence of different types of skin lesions. Alcoholic extracts of wheat and maize were potent in curing the skin symptoms of the florid (*a*) type developed in the absence of vitamin B₆ [see Chick *et al.*, 1935].

Considering the dietetic importance of cereals, the connection of endemic pellagra with the consumption of maize and the theories connecting the etiology of this disease with deficiency of vitamin B₂, it seemed of interest to investigate more fully the dietary factors present in an 80 % alcoholic extract of maize and other cereals, and the relative amounts of the two components of vitamin B₂ present in whole maize and wheat and their milled products.

METHODS.

Alcoholic extracts of whole wheat, white patent flour, whole maize, maize endosperm (polenta) and ground polished rice were prepared according to the method of Bourquin and Sherman.

800 g. of the cereal were shaken at room temperature (*ca.* 18°) for 1·5 hours with 1·5 litres of 80 % (by weight) alcohol, and the extract filtered through a Büchner filter with suction. The residue was then shaken for 1 hour with a fresh litre of 80 % alcohol, again filtered and the residue washed with about 300 ml. of 80 % alcohol. The combined filtrates, concentrated *in vacuo* to about one-fourth of their original volume, were then poured on to 300 g. of corn starch and dried at

room temperature under an electric fan. Thus 300 g. of the final prepared starch contained the extract from 800 g. of the original cereal. In the preparation of the extract, allowance was made for the water content of the cereals, the first extraction usually being made with 85% alcohol. Diets were prepared, of which 100 g. contained the extract from 50 g. cereal (as in the original specification of Bourquin and Sherman) and from 150 g. of cereal, respectively. These diets may then be stated to contain 50 and 150 "gram equivalents" of cereal %, respectively. For convenience, they are called Diets B and S I and B and S III and their compositions are given below.

	Diet B and S I	Diet B and S III
Caseinogen	18	18
Salt mixture	4	4
Hardened cottonseed oil	8	8
Lard	2	2
Corn starch	49	12
Corn starch + cereal extract	19	56

Young rats which had been partially deprived of vitamin B₂ from birth, as described by Chick *et al.* [1935], were weaned at 21 days, when they weighed 30–40 g., placed in separate cages and given the above diets containing cereal extracts, together with vitamins A and D as a separate dose of cod-liver oil. Rats receiving the B and S I diets were given additional vitamin B₁ as Peters's concentrate. Otherwise, when they ate very little, it was feared that they might be short of this vitamin. It was assumed that rats receiving B and S III diets obtained enough vitamin B₁ except when the diets contained extracts of highly milled cereal products; in this case a supplement of vitamin B₁ was given.

INVESTIGATION OF ALCOHOLIC EXTRACTS OF CEREALS FOR THE PRESENCE OF VITAMIN B₆ AND FLAVIN.

(a) Incidence and cure of skin symptoms in rats receiving diets containing wheat extract.

In an initial experiment to investigate the alcoholic extract of wheat, a litter of 9 young rats was used; 5 rats received the B and S I, and 4 the B and S III type of diet. After 3–5 weeks all the animals showed a severe degree of the (b) type of generalised skin affection, which has been shown to be developed in the absence of flavin when vitamin B₆ is provided.

Of the 5 rats receiving the B and S I diet, 3 which had ceased to grow and had developed severe skin symptoms were dosed with hepaflavin (12 γ) daily for 4 weeks, during which period their skin lesions were healed and there was an average gain in weight of 8 g. weekly. One of the other two rats on this diet was given a dose of vitamin B₆ in the form of a yeast extract from which the vitamin B₁ and flavin had been removed by autoclaving and treating with fuller's earth [see Chick *et al.*, 1935], but this supplement caused no improvement of the severe (b) type of skin symptoms, nor was growth restored until flavin was given as well. The condition then improved rapidly and the rat gained an average of 18 g. weekly for 4 weeks on the combined dose. The fifth animal was in a very poor condition and died a few days after it was given a dose of flavin, without showing any remission of its symptoms.

The 4 rats which received the B and S III diet remained in a better condition than those receiving the B and S I diet and even grew slightly for 2 or 3 weeks. They also developed severe (b) type of skin symptoms after 6–10 weeks and 3 were given daily doses of 12 γ flavin. Their average weekly gain in weight was 18 g. for 4 weeks and their condition was good at the end of the experiment. The fourth animal of this group received a dose of vitamin B₆ for 3 weeks, during which it

showed no improvement and its condition became very bad. Improvement took place after an additional dose of 12 γ of flavin was given, but growth was sub-normal (average of 10 g. weekly) and the skin symptoms did not clear so quickly as those of other rats which had been deprived of flavin for shorter periods.

This supplementary effect of flavin added to a diet containing wheat extract, in curing the generalised skin condition developed on this diet, indicated that the alcoholic extract of whole wheat contained a fair amount of vitamin B₆ but was deficient in flavin.

(b) *Effect on growth of extracts from different cereals.*

With weight increase as criterion, a long series of experiments was made to study the vitamin B₂ components present in diets containing extracts of wheat, white top patent flour, maize, maize endosperm, ground polished rice. The diets were prepared to carry extracts supplying either 50 (B and S I) or 150 (B and S III) g. equivalents of whole cereal per 100 g. of diet, but for the milled cereal products, only diets carrying 150 g. equivalents were used, as it was thought probable that their content of vitamin B₆ would be much less than that of the corresponding whole grains.

Young rats given these diets at weaning sometimes grew slightly for 2 to 3 weeks, but their weight generally remained stationary at about 50 g. and they rapidly developed skin symptoms of the (b) type. Out of 42 rats which received these diets for 6 weeks, 37 showed the (b) type of symptoms. After 3-6 weeks the rats were dosed with flavin and the growth response to 12 γ daily of flavin for 4 weeks was observed. The skin symptoms cleared up in every case in which they were present. The results are summarised in Table I.

Table I.

Group	Cereal diet	No. of rats	Before flavin doses			After flavin doses		
			Food intake		Av. weekly increase in wt. g.	Food intake		Av. weekly increase in wt. g.
			g. per week*	g. equiv. of cereal per week*		g. per week	g. equiv. of cereal per week†	
1	B and S I wheat extract	7 (a)	38	19	2	30	15 (11-18)	7
		5 (b)				52	26 (21-32)	13
2	B and S III wheat extract	4 (a)	52	78	2.5	46	69 (55-78)	16
		3 (b)				60	91 (88-93)	19.5
3	B and S I maize extract	5 (a)	56	28	1.5	30	15 (13-19)	9
		6 (b)				52	26 (22-33)	13.5
4	B and S III maize extract	2 (a)	56	84	2.5	49	74 (69-77)	16
		7 (b)				58	89 (81-99)	15
5	B and S III white flour extract	8	36	54	2	52	77	11
6	B and S III polenta extract	11	29	44	1.5	50	75	11
7	B and S III ground rice extract	8	35	52	2	52	77	10

* The diets were fed as dry powders and at first there was some scattering so that the figures for intake in cols. 4 and 5 are to be regarded as approximate.

† Figures in brackets indicate the extent of variation within the group.

The variation in the intake of supplementary factors, vitamins B₁ and B₆, with variations in appetite, constitutes a fundamental difficulty in the use of the Bourquin and Sherman type of diet, but in these experiments the effect of any

variability in the amount of vitamin B₁ can be neglected, since the adequacy of the supply was assured by giving additional doses of Peters's vitamin B₁ concentrate from yeast in all cases where it was considered that the intake in the diet was low. The food intake was completely recorded in all experiments so that the amounts of vitamin B₆ ingested could also be checked.

It was found that intake and growth response varied considerably in individual rats, similarly treated, without relation to sex, so that the results were analysed on the basis of a division into groups (a) and (b) eating more or less than 20 g. equivalents weekly in the group receiving the B and S I type of diet, and more or less than 80 g. equivalents in the group receiving the B and S III diet. These amounts indicate not only the cereal extract consumed (and the vitamin B₆ consumption) but, in any one group, are also proportional to the calorie intake.

In the period before the diets were supplemented with flavin all the rats showed a uniform lack of growth whatever the intake of cereal extract; after flavin was given those rats receiving the B and S III diets showed much more satisfactory weight increases than those on B and S I diets. At the lower levels of intake there would not appear to be a great difference in effective supplementary factors in the extracts from wheat and maize, but at higher levels of intake wheat extract appears to be rather better than maize in growth-promoting effect. With wheat extract, increased intake in group 2b (Table I) is reflected in an increase in average growth rate, in group 4b with maize extract there is no such effect, and the possibility is suggested that the larger intake of the alcoholic extract of maize may even have had a slight depressing effect on growth.

Extracts from milled cereal products, when supplemented with flavin, elicited a much lower growth response in proportion to intake than the extracts from whole cereals. There is a striking agreement between the figures for intake and gain in weight in groups 5, 6 and 7, Table I, which indicates that the endosperm of these cereals contains a fairly constant, if somewhat low, amount of vitamin B₆. The average intake of the cereal extracts in these groups was about the same as that of the (a) groups receiving the extracts of whole wheat and maize in similar B and S III diets, but the growth response was much less. It must therefore be concluded that a considerable proportion of the vitamin B₆ present in the original cereal had been lost in milling and was therefore situated in the germ and integuments.

In some experiments the addition of extra vitamin B₆, in the form of a yeast extract from which vitamin B₁ and flavin had been removed, was investigated, but such addition did not usually improve the condition of the rats or increase the growth rate. This was to be expected as they were already receiving vitamin B₆ in the cereal extracts but were deficient in flavin.

It may be concluded from these experiments that the alcoholic cereal extracts are rich in vitamin B₆ and deficient in flavin. The latter observation is not unexpected as, even if flavin were present in the whole cereal, it would not easily be extracted by cold 80 % alcohol.

Comparison of whole cereals and alcoholic cereal extracts as regards content of vitamin B₆ and flavin.

Birch and György [1935] found that only part of the vitamin B₆ activity of wheat and maize was extracted by cold 80 % alcohol and the following tests with diets containing whole cereals and cereal extracts have confirmed this observation.

Diets containing 50 g. of whole wheat or maize per 100 g. of diet were compared with B and S I diets containing 50 g. equivalents of whole cereal per 100 g. of diet. A diet with white top patent flour was also made up to compare with the diet containing extract of flour (Table I). The composition of the diets, allowing for variations in the content of protein and carbohydrate in the cereal was as follows: caseinogen 11, salt mixture (McCollum's No. 185) 4, hardened cotton-seed oil 7, lard 2, corn starch 26, cereal 50.

Exp. 1. These diets were tested on 25 young rats from 3 litters, groups of 5 receiving 50% whole wheat diet, B and S I wheat extract diet, 50% whole maize diet, B and S I maize extract diet and 50% whole flour diet, respectively (see Table II).

Table II.

Group	Cereal diet	Before flavin doses				After flavin doses			
		No. of rats	Time in weeks	Weekly intake of cereal, g. or g. equiv.	Av. weekly increase in wt. g.	No. of rats	Time in weeks	Weekly intake of cereal, g. or g. equiv.	Av. weekly increase in wt. g.
Exp. 1:									
1	Whole wheat 50%	4	4	32	13.4	1	4	43	20
2	White flour 50%	5	4	14	1.0	5	4	21.9	10
3	Whole maize 50%	5	4	22	6.0	4	4	33	16.4
4	B and S I wheat extract	5	4	13	-0.3	4	4	20.4	9.0
5	B and S I maize extract	5	4	13	0.2	5	4	20	9.0
Exp. 2:									
6	Whole wheat 30%	4	2-4	7.5	7.2	4	4	22	22
7	Whole maize 30%	4	2-4	11	6.1	4	4	21	16.5
8	B and S I wheat extract	4	2-4	15	4.8	4	4	26	12.7
9	B and S I maize extract	4	2-4	16	4.6	4	4	28	14.5

The rats receiving the diet containing 50% whole wheat grew steadily at a rate of 10-19 g. weekly without additional flavin, except in the case of 1 rat which ate very little in the first 2 weeks of the experiment and did not thrive until it received a dose of flavin, after which it grew 81 g. in 4 weeks. The 5 rats receiving the diet containing 50% whole maize showed a much smaller increase in weight, only about 6 g. weekly, until they received additional flavin, when their growth rate was of the same order as that of their litter-mates receiving the whole wheat diet without added flavin. The results were complicated by differing food consumption, since the rats receiving the 50% whole wheat diet ate 23-45 g. wheat per week, and those receiving the 50% whole maize diet ate only 16-29 g. of maize per week before receiving the flavin doses but 20-40 g. afterwards.

The rats in groups 4 and 5 receiving diets containing 50 g. equivalents per 100 g. of wheat and maize as extract were all given flavin doses at the same time as litter-mates in group 3 receiving the 50% whole maize diet, and their average growth was much less than that of the rats receiving the unsupplemented whole wheat diet, or those receiving the whole maize diet with additional flavin. Those receiving the wheat extract diet supplemented with flavin had an average intake of 20 g. equivalents of wheat weekly and only showed an average gain of 9 g. weekly, and those receiving the maize extract diet showed similar figures for both intake and growth. The amounts of extract consumed in groups 4 and 5 were not equivalent to the amounts of whole cereal consumed by rats in groups 1 and 3 receiving the whole cereal diets, but even so the growth response was so much less as to suggest that by no means all the vitamin B₆ of wheat and maize is extracted by 80% alcohol at room temperature.

The results of this experiment did not give a quantitative estimate of the extractibility of the vitamin B₆ in wheat and maize by cold 80% alcohol, but did

serve to demonstrate a notable difference between whole wheat and whole maize for the maintenance of growth and good condition and indicated the presence of the whole vitamin B₂ complex in whole wheat, but a lack of the flavin component in whole maize.

Rats receiving the diet containing 50 % white flour, before they were given flavin, grew rather less than those receiving the whole maize diet and very much less than those receiving the whole wheat diet; their intake was, however, very low. After they were given doses of flavin their average weekly growth was 10 g. and average intake 22 g. of flour weekly. A comparison of these figures with those for group 5 in Table I shows a very similar growth result (weekly average 11 g.) for rats which ate 77 g. equivalents of flour as alcoholic extract, thus indicating that the alcoholic extract contained only one-third to one-quarter of the vitamin B₆ present in the flour.

Exp. 2. An experiment was made with diets containing 30 % whole wheat and maize, in the hope of obtaining a better estimate of the vitamin B₆ content of these grains and of the extent to which it was extracted by alcohol. The results given in Table II show that in the case of wheat supplemented with flavin, with comparable intake of whole cereal and of g. equivalents of cereal extract (22–26 g. or g. equiv.), the growth with the former (weekly average 22 g.) far exceeds that with the latter (13 g.). A comparison of these figures with those of groups 1 (b) and 2 (b) in Table I suggests that such a difference in growth response corresponds to about three times the intake of cereal as g. equivalents, and if this calculation be permitted, it may be estimated that rather less than one-third of the vitamin B₆ in whole wheat is extracted by cold 80 % alcohol.

The maize extract contained definitely less vitamin B₆ than an equivalent amount of whole maize, but no exact calculation of the proportion extracted could be made as there was no consistent increase in growth effect corresponding to increased intake either of the whole cereal or of the extract.

Further, this experiment provides more evidence of the fact that wheat contains more flavin than maize.

CONCLUSIONS.

These results confirm the observation of Birch and György [1935] that cereals are a good source of vitamin B₆. Wheat and maize seem to be about equally potent sources at levels of intake up to about 20–30 g. or g. equiv. of cereal weekly. At higher levels of intake there is evidence that maize was either a less effective source of vitamin B₆, or that its activity was prejudiced by the presence of some other factor, possibly harmful to rats. For whilst increased intake of wheat, either as such or as the 80 % alcoholic extract, was accompanied by increased growth, a similarly increased intake of maize only allowed increased growth up to a limited amount, beyond which there was no further increase and sometimes even a slight falling off. Similar results were obtained with all types of maize diet used, whether containing whole maize or the extract (see Table I, groups 3 and 4; Table II, groups 3 and 7), so that if there is some deleterious factor in maize it would seem to be extracted by cold 80 % alcohol.

The milled products of wheat and maize, and also ground polished rice, were found to be less active sources of vitamin B₆ than the whole cereals. A comparison of groups 5 and 6 in Table I with groups 1 and 3, respectively, suggests that flour and polenta contain only about one-third of the amount of vitamin B₆ present in the whole grains, since when supplemented with flavin about three times the intake (calculated as g. equiv.) is needed to support a similar growth rate. In

the case of flour this result agrees with that derived from a comparison of the unextracted materials, which showed that white flour was much inferior to whole wheat as a source of vitamin B₆. It is therefore apparent that much of the vitamin B₆ of the grain is contained in the germ and integuments.

Whole wheat was also found to be a slightly better source of the entire vitamin B₂ complex than whole maize, but when the whole maize diets were supplemented with flavin the growth rate became approximately equal to that obtained with whole wheat unsupplemented. Whole wheat would therefore appear to contain a greater amount of flavin than whole maize. This conclusion has been confirmed by Dr Ellinger (private communication) by chemical work, in which an attempt was made at quantitative extraction and estimation of flavin in wheat and maize.

The experiments made with the Bourquin and Sherman diet indicated that the flavin of cereals was not extracted with cold 80 % alcohol, but that about one-third to one-quarter of the vitamin B₆ could be removed by two extractions with cold 80 % alcohol. This finding is not in accord with that of Birch *et al.* [1935, pp. 2836, 2849] who found this vitamin to be extracted with great difficulty.

A significant nutritional difference has thus been demonstrated between wheat and maize in the larger content of flavin in wheat. The experiments in this paper permit no other conclusion. The result is, however, opposed to that of Birch *et al.* [1935] who found flavin present in maize in greater amount than in wheat. We can suggest no explanation for this discrepancy unless there is considerable variation in the flavin content of different samples of maize.

It is important to note that the use of the Bourquin and Sherman type of diet in investigations of this type is not very satisfactory, since the variation in intake of food with the appetite of the experimental animals also causes variation in the intake of certain essential dietary factors and makes results more difficult of interpretation. A more reliable result would be obtained by giving the test materials as separate doses, as in a few experiments reported elsewhere [Copping, 1936].

PELLAGRA AND MAIZE-EATING.

It would be tempting to emphasise the significance of the deficiency of flavin in maize, seeing that many substances which prevent and cure pellagra (liver, liver extract, meat, milk, yeast) are rich in flavin. Clinical trials have not, however, indicated any curative property for human pellagra in pure flavin [Dann *et al.*, 1935; Spies and Chinn¹] nor can black tongue in dogs be cured by it [György, 1935]. Further, white flour, on which so many wheat eaters subsist, is equally deficient in flavin and populations living in poverty in isolated districts and subsisting too exclusively on milled wheaten products tend to develop beriberi, whilst endemic pellagra is unknown in such circumstances [Aykroyd, 1930].

There is, however, in some of the experiments recorded in this paper a suggestion of an unwholesome element in maize. In several tests, increasing the amounts of maize or maize extracts in the diet, even when these were providing an essential vitamin, did not show an increasing beneficial effect, as was the case in similar trials with wheat and wheat extracts. The theory postulating some toxic factor in maize and regarding endemic pellagra as a disease of intoxication has been put forward by many investigators, chiefly on clinical grounds, and is possibly supported by this observation.

¹ See Birch *et al.* [1935, p. 2850].

SUMMARY.

1. The results of experiments on rats with diets of the Bourquin and Sherman type, containing extracts of wheat or maize or their milled products, or diets containing the unextracted whole cereals, indicate that wheat and maize are good sources of vitamin B₆ and that one-third to one-quarter of the vitamin B₆ present is extracted by cold 80 % alcohol.
2. Wheat appears to contain a greater amount of flavin than maize, but no flavin is extracted by cold 80 % alcohol.
3. The vitamin B₆ in both wheat and maize appears to be present in greater amount in the germ and integuments than in the endosperm.
4. Some of the experiments with maize and maize extracts suggest the possibility that a deleterious substance is present in this cereal, and is, moreover, extracted by cold 80 % alcohol.

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