CLXII. THE VITAMIN B₂ CONTENT OF CEREALS AND THE SUPPOSED CONNECTION BETWEEN HUMAN PELLAGRA AND DEFICIENCY OF THIS VITAMIN.

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(Received August 28th, 1930.)

IN a recent paper [Aykroyd and Roscoe, 1929] are given the results of an examination of cereals and other foodstuffs for their vitamin B₂ content, using a method of estimation previously described by Chick and Roscoe [1928]. Vitamin B₂ was defined as "the factor in the vitamin B complex other than the antineuritic, which promotes growth and prevents dermatitis in rats." The same definition holds good here. In the previous paper a summary of the literature on the subject was given, and nothing has since been published relative to the distribution of vitamin B₂ except an observation of Axtmayer [1929], that kidney beans can supplement autoclaved yeast (vitamin B₂) fed as sole source of the vitamin B complex, while polished rice supplements neither autoclaved yeast nor beans and is presumably lacking in both factors.

Aykroyd and Roscoe showed that the distribution of vitamin B₂ corresponds with that of the P-P factor of Goldberger and his co-workers [Goldberger *et al.* 1925, 1926], held to be preventive and curative of human pellagra, and also with that of the dietary factor which prevents "black-tongue" in dogs [Goldberger *et al.* 1928 1, 2]. The rat's needs for vitamin B₂ were found to be satisfied by the various foodstuffs in the order of their potency as regards the P-P factor and the black-tongue-preventive factor. It was, therefore, suggested that these three factors are identical; and that the dermatitis frequently produced experimentally in vitamin B₂-deficient rats is the analogue of human pellagra, which in some ways it resembles, and of black-tongue. At the same time it was pointed out that rat "pellagra" does not develop on diets which produce human pellagra and black-tongue; a much more highly purified ration is required. It was found impossible, for example, to produce rat "pellagra" by feeding with maize.

The purpose of the present research was to investigate further the relation between the epidemiology of human pellagra and vitamin B₂ deficiency. One of the most striking (and hitherto unexplained) facts about human pellagra is

its almost exclusive occurrence among maize-eating populations. In the previous experiments [1929] we found that whole maize, though low in vitamin B₂ when compared with foods containing animal protein, was by no means devoid of the vitamin, and that maize endosperm compared well with wheat flour in this respect. In the present research two other staple cereals, rice and millet, have been included and their vitamin B₂ content compared with that of maize.

METHOD USED FOR ASSAYING VITAMIN B₂.

The technique employed was that used previously [Chick and Roscoe, 1928; Aykroyd and Roscoe, 1929]. Vitamin B₁ is supplied to the rats as Peters's antineuritic concentrate [Peters, 1924; Kinnersley and Peters, 1925], 0.1 cc. daily (equivalent to 0.6 g. yeast) being more than sufficient to cover the young rat's needs for this vitamin. The caseinogen in the basal diet is freed as far as possible from vitamin B₂ by repeated washing with acidulated water and extraction with dilute acid alcohol. The composition of the basal diet (P₂L) is as follows:

Specially purified caseinogen	...	...	20 parts
Rice starch	...	...	60 „
Arachis oil	...	...	15 „
Salt mixture	...	...	5 „
(Water	...	...	100-130 „)

The diet is cooked in a steamer for 3 hours at 100° to prevent complications due to "refection" [Fridericia *et al.* 1927; Roscoe, 1927].

Previous experience suggested that large daily amounts of cereal foods are required to supply vitamin B₂. These foodstuffs, therefore, were not fed separately from the basal diet, but this diet was modified so as to include the substance under examination, the ratio (20 : 60 : 15) between protein, carbohydrate, and fat being approximately maintained. The analyses of the cereals in a standard work [Tibbles, 1912] were used for the calculation. For example, the diet including 65 % of milled rice was constructed as follows, the composition of rice being taken as protein 7 %, carbohydrate 93 %, fat negligible.

Caseinogen	...	...	13.5 parts
Rice starch	...	...	0 „
Arachis oil	...	...	15 „
Salt mixture	...	...	5 „
Milled rice	...	...	65* „
Water	...	...	100 „

* 5 parts protein, 60 parts carbohydrate.

The composition of other cereal diets was calculated in the same manner. In no case did cereal protein replace more than one-half of the caseinogen in the original basal diet; in most diets it replaced about one-third. The diets were cooked for 3 hours at 100°.

Young rats, just weaned, from 35 to 45 g. in weight, were fed for a week on the basal diet P₂L, with a daily ration of 0.05–0.01 g. cod-liver oil to provide vitamins A and D, Peters's antineuritic concentrate being omitted. During this period no growth took place. At the end of the week the rats were put in separate cages and the material under examination fed to them, 0.1 cc. (= 0.6 g. dry yeast) daily of Peters's antineuritic concentrate being administered to each separately to supply vitamin B₁. The best grown rat from each litter was kept on the basal diet P₂L with additions only of cod-liver oil and the antineuritic concentrate to act as a "negative control."

The test diets were fed to the rats for 5 weeks. The first week, during which the animals were under strange conditions and consuming a strange diet, was disregarded and the vitamin B₂ value of a substance was estimated by its power to produce growth during the subsequent 4 weeks. As a rule the materials were tested on rats from three different litters, and in no case were conclusions drawn from results obtained on rats of one litter only. Males and females were used in approximately equal numbers.

THE VITAMIN B₂ CONTENT OF WHOLE MILLET (*SORGHUM VULGARE*), WHOLE RICE, MILLED "RAW," MILLED "PARBOILED" RICE, WHOLE MAIZE AND MAIZE ENDOSPERM.

A sample of millet was procured from the Sudan, where millet is the staple cereal. Two samples of whole rice, Japanese (A) and Burmese (B), were examined. The specimen of "raw" milled rice was of the same origin as B; it was not highly milled or polished and represents a "poorer" commercial grade of rice than any sold in the European markets, and one typical of that eaten by the townsfolk of the tropics. The "parboiled" rice was obtained from British Guiana. The "parboiling" process consists in soaking rice in water for 24–36 hours, steaming for about 10 minutes, with subsequent drying in the sun for about 12 hours, an operation designed to make easier the removal of the husk. "Parboiling" is usually employed in countries and districts where modern milling machinery, capable of dealing with raw or untreated rice, is not available. The process renders the grain less friable and makes it keep better [Douglas, 1930]. The sample tested had lost in milling the germ and most of the pericarp. "Parboiled" rice, subjected to various degrees of milling, forms the staple food among many communities [Fraser and Stanton, 1911; Douglas, 1930].

In addition, samples of maize endosperm were fed to rats as 65 % of the basal diet, in the hope that "pellagra-like" symptoms might develop. One of these, "white granulated hominy," obtained from the Quaker Oats Co., Ohio, is of a type widely sold in the pellagra districts of the Southern United States.

For purposes of comparison, some results previously published are included in Table I.

Whole millet is a poor source of vitamin B₂: 50 % in the diet produces only

an average weekly increment of 8 g., the normal increase in body-weight being at least 11 g. weekly, a figure sometimes greatly exceeded. Similarly, all the rice samples examined, milled or unmilled, are very poor in the vitamin. The low vitamin B₂ content of millet and rice may be estimated by a comparison with yeast. 0.2 g. daily of dried yeast (about 2.5 % of the food consumed) is sufficient, as a supply of vitamin B₂, for normal growth.

Table I. *Vitamin B₂ content of various cereals and cereal products.*

Average weekly growth of rats on: I, (a) purified diets deficient in vitamin B₂, and (b) with optimum amount of vitamin B₂ supplied by autoclaved yeast; II, synthetic diets in which vitamin B₂ is supplied by cereal products in various percentages. (Vitamin B₁ is supplied by a daily dose of Peters's antineuritic concentrate.)

Material tested							No. of rats observed	Average weekly increase in body-weight for chosen 4 weeks (g.)
I. (a) Negative controls (diet P ₂ L)							12	3
(b) Positive controls* (diet P ₂ L) + autoclaved yeast (0.4 g. daily)							5	12.5
II. Millet:								
Whole ground millet 30 %							4	5
" 50 %							6	7
Rice:								
Whole ground rice (Japanese) 30 %							2	2
" " 50 %							5	5
" (Burmese) 50 %							4	6
Milled rice (raw) (Burmese) 65 %							4	4
" (parboiled) (British Guiana) 65 %							6	5
Wheat:								
Whole ground wheat, Manitoba 50 %*							10	16
" England 50 %*							4	12
Flour: 65 %*							11	5
Maize:								
Whole ground maize. White African 65 %*							6	11
" Yellow S. American 65 %*							8	8
Endosperm. Maize grits (S. American) 65 %*							6	5
" Yellow maize flour (U.S.A.) 65 %							6	5
" "White granulated hominy" (U.S.A.) 65 %							4	6

* [Aykroyd and Roscoe, 1929.]

Of the whole cereals tested, wheat has the highest vitamin B₂ content. Maize comes next, then millet, and lastly rice, which is very low in vitamin B₂. Rats on the 50 % whole rice diet, or the 65 % milled rice diet, grew very little more than the negative controls on the unsupplemented basal diet P₂L. The cereals as a class are poor sources of vitamin B₂, and wheat, the most potent so far discovered, is low compared with other foodstuffs [Aykroyd and Roscoe, 1929]. It is important to notice that maize is a rather better source of vitamin B₂ than millet or rice.

The endosperms of wheat, maize and rice are all about equally low in the vitamin; very poor growth was attained on diets containing 65 % of either. No "pellagra-like" symptoms were observed on any of the cereal diets.

Negative controls.

In the previous experiments 60 % of the animals kept as "negative controls," *i.e.* on diet P₂L, with the addition only of cod-liver oil and the anti-neuritic concentrate, developed symmetrical dermatitis. In the present experiments there has been more irregularity in the occurrence of skin symptoms. Of 11 negative control rats kept on the basal diet for over 12 weeks, 2 developed, in 11 and 16 weeks respectively, symmetrical raw areas on the skin, in one case on the face and in the axillae, in the other on the inside of the thighs. Three other rats died without marked skin symptoms in 11–14 weeks. These animals all showed an unhealthy, mangy appearance, with loss of hair round the eyes, followed by swelling and inflammation of the lids.

The vitamin B₂-deficient animals have shown a fairly consistent symptomatology, but the striking symptom previously recorded [Chick and Roscoe, 1927], florid, oedematous dermatitis of the fore-paws appearing in 6 to 8 weeks, has not been observed. Three rats survived for 18–24 weeks without developing characteristic dermatitis, the remaining 3, also unaffected by dermatitis, were observed for a shorter period. Most of these showed, in addition to stunted growth and a very poor condition of the fur, a symptom common to the more adversely affected negative control animals, which has been called "haematuria" in previous papers from this laboratory. A reddish-brown stain appears round the urethra after the animal has been about 8 weeks on the vitamin B₂-deficient diet. It has been found that this stain, which rapidly disappears when adequate amounts of vitamin B₂ are fed, does not give the benzidine tests for blood, and at present its exact nature is unknown. In addition, most of the negative control rats have shown blood-stained wrists, pointing to discharge from the nose and eyes.

The basal diet has not been substantially altered, purification of the caseinogen having been carried out in as thorough a manner as formerly, and the negative control animals show a failure to grow, similar to that observed in earlier experiments. If the relative absence of severe dermatitis is due to the basal diet containing more vitamin B₂, this excess does not produce any significant increase in body-weight. It is possible that an alteration in the "vitamin B" content of the breeding diet, a change from marmite to dried brewer's yeast, has been responsible. Whatever the explanation, it is disconcerting to find that typical rat "pellagra" is not consistently developed on a highly purified food mixture, apparently complete except for vitamin B₂, which formerly induced severe skin symptoms regularly in 6 to 10 weeks.

Since so few rats developed characteristic symptoms, only a small number of curative tests for rat dermatitis were carried out (Table II).

Neither maize endosperm nor whole rice had any curative effect on the symptoms produced by vitamin B₂ deficiency. Dermatitis in both rats actually became prominent while maize was being fed, or just after, but the animals were on the verge of dermatitis before the curative test was started.

Table II. *The curative effect of certain substances on the dermatitis and other symptoms developing in rats on a vitamin B₂-deficient diet (P₂L).*

Rat	Condition before test	Substance tested	Length of time fed (weeks)	Result
895 ♀	Wretched condition. Inflamed eyelids. Urine red-stained. General loss of hair. Weight stationary	White hominy grits (maize endosperm) 65 % in diet	2	Condition made rather worse. No increase of weight
	—	Whole rice (Burmese) 50 % in diet	2	Condition definitely worse. Dermatitis of face and axillae developed
	—	P ₂ L + 1 cc. yeast fraction (autoclaved) = 5 g. dry yeast	3	All lesions healed. 23 g. increase in weight
917 ♂	Wretched condition. Inflamed eyelids. Red-stained urine. Loss of hair. Diarrhoea. Weight stationary	White hominy grits (maize endosperm) 65 % in diet	2	Condition worse. Dermatitis developed on face and inner surface of thighs
	—	Whole rice (Burmese) 50 % in diet	2	Animal became rapidly worse and died

DISCUSSION OF THE RELATION OF VITAMIN B₂ TO HUMAN PELLAGRA.

The close association between pellagra and the consumption of maize has been so widely recognised for over a century as scarcely to need re-stating. It is a fact which any dietetic theory of the origin of pellagra must take into account.

Maize-eaters do not appear to suffer from beriberi, a disease which evidence shows is due to deficiency of vitamin B₁. Beriberi has not been reported among the maize-eating populations of Italy, Egypt and certain States in the American Union. This is probably due to the fact that maize is often consumed "whole" or undermilled, *i.e.* is not deprived of its vitamin B₁ content by complete removal of the embryo and integuments. It might be argued that the immunity of the maize-eater to beriberi is due to the state in which maize is eaten or to other dietary factors and enables him to subsist long enough on the defective cereal to develop pellagra, supposing the latter disease to be due to deficiency of vitamin B₂ and to need a long depletion period. Whole maize is by no means devoid of vitamin B₂ but is a poor source compared with many other foodstuffs [Aykroyd and Roscoe, 1929]. An adequacy of vitamin B₁, coupled with a lack of vitamin B₂, is a necessary factor for the development in the rat of the symptoms of vitamin B₂ deficiency. On these lines might be explained the fact that the consumer of milled rice, who is liable to beriberi, rarely succumbs to pellagra, though milled rice has been found to contain no more vitamin B₂ than the maize products examined.

The writer [1930] has recorded that Newfoundland fishermen, who sometimes subsist for many months on a diet mainly composed of refined wheat flour, a foodstuff low in both B vitamins, develop beriberi but not pellagra.

Their immunity to the latter disease might similarly be due to their earlier liability to beriberi.

This reasoning would, however, fail to explain why rice so prepared that it is protective against beriberi, *i.e.* undermilled, or "parboiled"¹ [Fraser and Stanton, 1911], is rarely associated with pellagra, since neither "whole" nor "parboiled" rice shows any superiority over maize as regards vitamin B₂ potency. The occurrence of pellagra on rice has been recorded [Stannus, 1912, 1913; Sheppard, 1912] but these instances stand alone in the literature. The enormous rice-eating communities of India and the East, many of whom eat rice in the parboiled state [McCarrison, 1924], do not appear to suffer from pellagra. If pellagra is due to vitamin B₂ deficiency the consumer of "parboiled" or undermilled rice should be as liable as the maize-eater.

Again, there is no marked difference between the vitamin B₂ value of whole maize and whole millet. The latter cereal contains vitamin B₁ [Plimmer *et al.*, 1927] and is not commonly associated with beriberi. For the sample of millet tested, I am indebted to Colonel Archibald, Director of the Gordon Research Institute, Khartoum, who has also very kindly contributed the following information about native dietaries in the Sudan. The Arab tribes in that country can obtain, in addition to millet, milk, vegetables, meat and fish, but the Negro tribes supplement the millet only with "vegetables in small quantities and butcher's meat occasionally." Their diet consists in the main of "kisra," fermented millet dough, and "merissa," a native liquor made from the same cereal. Nevertheless Colonel Archibald states that pellagra in the Sudan is unknown. Pellagra on a staple of millet has been recorded [Wilson, 1921], but only in an isolated instance. If pellagra were due to vitamin B₂-deficiency the negro population in the Sudan should be as liable to the disease as the consumers of whole maize elsewhere.

It is difficult to imagine that maize-eaters regularly consume fewer dietary accessories than rice or millet eaters. There are numerous poor populations who live on a staple diet containing little apart from rice or millet.

At present the association, if any, of vitamin B₂ and human pellagra is obscure. The general distribution of vitamin B₂ corresponds well with that of Goldberger's P-P (pellagra-preventive) factor, and the black-tongue-preventive factor [Aykroyd and Roscoe, 1929], as far as all three are known, and with the foodstuffs known from clinical observation to be preventive and curative of human pellagra. Nevertheless, the distribution of vitamin B₂ as worked out on the rat, leaves the association of pellagra with maize unexplained. It is conceivable that a positive, toxic, "pellagra-producing" factor, operative only

¹ The parboiling process is supposed to toughen the grain and render the removal of the pericarp more difficult in the subsequent milling [Fraser and Stanton, 1911]. Whatever the reason, there is good evidence that parboiled rice usually contains enough vitamin B₁ to protect against beriberi. The specimen of milled parboiled rice tested in these experiments for vitamin B₂ was also examined for vitamin B₁. As compared with a sample of "raw" rice of the same origin and apparently about equally milled, it was found to be an adequate source of the antineuritic vitamin (vitamin B₁). It is hoped to record these observations later.

in the absence of foods containing abundant vitamin B₂, exists in maize, and occasionally in other cereals, but of this there is so far no satisfactory evidence. Much effort with inconclusive results was made in the last century to discover a poisonous substance in good and "spoiled" maize.

THE RESULTS OF EXPERIMENTS TO DISCOVER A PHOTO-SENSITISING
SUBSTANCE IN MAIZE.

It is well known that the skin lesions of pellagra usually appear in parts of the body exposed to the sun's rays. Horbaczewski [1910, 1912] reported experiments demonstrating the presence in maize of a photo-sensitising substance. White mice fed on maize polenta and milk developed dermatitis, when exposed to sunlight, in periods ranging from 2 weeks to 6 months. Dermatitis was recorded in a number of rats subsisting on a diet of wheat flour and milk to which an alcoholic extract of maize was added. Subcutaneous injection of maize extracts into mice resulted in death in 2 to 3 hours in animals exposed to light, while in the dark they survived for 20 to 30 hours.

It is difficult to assess the value of Horbaczewski's observations. His papers contain contradictions and some experiments appear to be insufficiently controlled. Attempts to perform experiments on rats on lines similar to those of Horbaczewski have up to the present produced only negative results. Two albino and two black and white rats fed with Horbaczewski's milk and polenta diet, and exposed daily for 3 minutes to a mercury vapour lamp at a distance of 625 cm., thrived for 10 weeks, growing at a rate little short of normal.

The possibility was investigated that the action of the hypothetical toxic or photo-sensitising substance in maize might be more apparent in animals deprived of vitamin B₂. Accordingly samples of (1) whole maize, (2) maize flour and (3) maize grits (endosperm), finely ground, were shaken with two changes of 50 % (by weight) alcohol for 5 hours at 37°, in an attempt to extract vitamin B₂. Narayanan and Drummond [1930] have recently shown that vitamin B₂ is soluble in 50 % alcohol. The extracted maize samples were incorporated as 65 % of a diet (P₂L) similar to that used for vitamin B₂ estimations, vitamins B₁, A and D being supplied as recorded previously. Four young piebald rats were placed on each of the 3 diets thus constituted, 2 being subjected to the mercury vapour lamp for 3 minutes daily. The extracted maize diets were low in vitamin B₂ and poor growth resulted, but no other recognisable ill effects were observed in 8 to 10 weeks.

In another experiment a 96 % alcoholic extract of whole yellow maize (the daily dose being equivalent to about 40 g. of the original maize) was fed to 3 black and white rats on a diet deficient in vitamin B₂. The animals showed some of the symptoms of vitamin B₂ deficiency but no obvious toxic effect due to the maize extract could be observed in 10 weeks in spite of daily exposure to ultra-violet light.

Horbaczewski's most striking results were obtained on white mice. It is felt that further work on mice is necessary to confirm his work or otherwise.

THE IDENTITY OF RAT "PELLAGRA" AND HUMAN PELLAGRA.

The distribution of vitamin B₂ in cereals gives no support to the theory that the dermatitis produced in rats by vitamin B₂-deficiency, originally described by Goldberger and Lillie [1926], is the analogue of human pellagra. The irregularity in the appearance of the "pellagra-like" symptoms and the impossibility of inducing them with diets containing maize raise a further barrier to the identification of the experimental condition with the human disease. It might be suggested that, apart from failure in growth, the rat is relatively insensitive to the effects of vitamin B₂-deficiency, but this would not hinder differences in vitamin B₂ value between maize and other foodstuffs being demonstrated by growth tests. It is at this point that the identification of vitamin B₂ and the P-P factor fails.

Whether, apart from its questionable association with pellagra, vitamin B₂ has an important rôle to play in human nutrition is at present uncertain. Populations who eat a diet largely cereal and particularly those subsisting on rice might be expected to suffer from deficiency of the vitamin, which is found in more abundance in expensive foods such as milk, meat and eggs.

SUMMARY.

1. Using Chick and Roscoe's method, the examination of foodstuffs for vitamin B₂ previously recorded has been further extended.
2. Two samples of whole rice and two samples of milled rice "raw" and "parboiled," were found to be poor sources of vitamin B₂.
3. Whole millet is a poor source of vitamin B₂.
4. Control rats from each litter were fed on the basal diet complete except for vitamin B₂. All remained practically stationary in weight, but skin symptoms did not consistently develop. Curative tests were performed on 2 rats suffering from dermatitis. Diets containing 65 % of maize endosperm and 50 % of whole rice respectively did not alleviate the symptoms.
5. It is pointed out that, since neither rice nor millet shows any superiority over maize as a source of vitamin B₂, it is difficult to accept vitamin B₂-deficiency as the sole cause of human pellagra, as this disease is almost invariably associated with the consumption of maize.
6. No satisfactory evidence has so far been obtained of the presence of a toxin in maize.

I am indebted to Messrs Carbutt and Co. for supplying samples of Burmese rice; and to Mr C. E. Douglas for his assistance in obtaining from the Department of Agriculture, British Guiana, a number of rice samples. The specimen of Japanese whole rice was obtained from the Orzone Co., Ltd. Free samples of maize from the Quaker Oats Co., and Messrs R. W. Paul, Ltd., are also acknowledged.

I am grateful to Colonel Archibald, of the Gordon Institute, Khartoum,

for samples of millet and some very interesting information about native dietaries.

Thanks are due to Miss Gaffikin for help in feeding the animals, and to Dr Chick and Sir Charles Martin for much useful advice.

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