

## CXXIII. THE WATER-SOLUBLE B-VITAMINS.

### V. NOTE ON THE TWO TYPES OF SKIN LESION OCCURRING IN VITAMIN B<sub>2</sub> DEFICIENCY IN THE RAT IN RELATION TO DEFICIENCY OF FLAVIN AND VITAMIN B<sub>6</sub>, RESPECTIVELY.

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IN recent papers György [1934; 1935] has shown the relation of the two constituents of vitamin B<sub>2</sub>, flavin and vitamin B<sub>6</sub>, to the skin lesions seen in rats receiving diets deficient in vitamin B<sub>2</sub>. Chick *et al.* [1935] also described two kinds of skin symptoms:

(a) A symmetrical florid dermatitis, with redness, swelling and oedema affecting first the paws and tips of ears and nose. This condition is similar to the "specific type" of dermatitis described by György and the pellagra-like condition in rats described by Goldberger and Lillie [1926].

(b) An affection of the skin in which the hair is shed without swelling or inflammation, with development of bald patches especially over the head and face; the eyelids are often stuck together with a serous, blood-stained fluid which exudes from the eyes and from the nostrils. This condition corresponds to that described as "non-specific" by György.

György found that vitamin B<sub>6</sub> prevented and cured the "specific", and flavin the "non-specific", skin lesions. Although in this laboratory we found that flavin cured the (b) skin affection, vitamin B<sub>6</sub> did not always cure the florid (a) type of dermatitis and we rather doubted whether the relation of the "pellagrous" rat dermatitis to lack of vitamin B<sub>6</sub> was as simple as György concluded [Chick *et al.*, 1935, pp. 730, 732].

It was, however, realised that the specificity of the two factors for these different skin affections could be better studied by observations of the prophylactic type on young rats maintained from the time of weaning on diets lacking only in flavin or only in vitamin B<sub>6</sub>. Subsequently attempts were made to cure the sick animals. These experiments, of which a short summary has already been published [Copping, 1935], form the subject of the present paper.

#### METHODS.

In previous studies [Chick *et al.*, 1935] on vitamin B<sub>2</sub>, a basal diet containing crude maize sugar as a source of carbohydrate produced skin symptoms more regularly than one containing rice starch. The following diet was therefore used: caseinogen 100, maize sugar 300, cottonseed oil 60, lard 15, salt mixture (McCollum's no. 185) 25. The diet was thoroughly mixed by hand and stored dry, but before feeding was made into a stiff paste with distilled water. Vitamins A, D and B<sub>1</sub> were given separately from dropping pipettes, A and D as cod-liver oil, and B<sub>1</sub> as Peters's concentrate from yeast, prepared according to the modified method described by Kinnersley *et al.* [1933]. The rats, which were deprived of

vitamin B<sub>6</sub> only, received 12γ of pure hepaflavin daily, and litter-mates, deprived of flavin only, received doses of vitamin B<sub>6</sub> as 1 ml. (equivalent to 0.5 g. of original dry yeast) of a yeast extract from which vitamin B<sub>1</sub> and flavin had been removed by autoclaving and treating with fuller's earth [see Chick *et al.*, 1935]. In some curative tests, vitamin B<sub>6</sub> was given as a cold alcoholic extract of whole wheat or whole maize, prepared according to the method described by Bourquin and Sherman [1931] for use in a modified Sherman and Spohn diet, in doses equivalent to 4 g. of original cereal [see also Copping, 1936].

Table I. *Type of skin lesions developed on "–B" diet with addition of vitamin B<sub>1</sub> and either flavin or vitamin B<sub>6</sub>.*

(a)=florid dermatitis; (b)=non-inflammatory skin affection with loss of hair; (0)=no symptoms.

| Group | Component of vit. B <sub>2</sub> given                                | No. of rats | Preparatory period   |     |     |         |               | Time in weeks | Av. weekly increase in wt. g.                                     | Curative period                    |             |               |                                  |        |
|-------|---|-------------|----------------------|-----|-----|---------|---------------|---------------|---|------------------------------------|-------------|---------------|----------------------------------|--------|
|       |   |             | Type of skin lesions |     |     |         | Time in weeks |               |   | Av. weekly increase in wt. g.      | No. of rats | Time in weeks | Av. weekly increase in wt. g.    | Result |
|       |   |             | (0)                  | (a) | (b) | (a)+(b) |               |               |   |                                    |             |               |                                  |        |
| A     | 0   | 3           | 0                    | 2   | 1   | 0       | 3–5           | –0.3          | (1) Purified vit. B <sub>6</sub> ≡0.5 g. yeast, dry wt.           | 1, (b)                             | 4           | 12            | Cure not quite perfect           |        |
|       |   |             |                      |     |     |         |               |               | (2) 12γ flavin + alcoholic extract of maize or wheat ≡4 g. cereal | 2, (a)                             | 4           | 16.5          | Perfect cures                    |        |
| B     | 12γ flavin  | 10          | 1                    | 8   | 0   | 1       | 4–14          | 3.4           | (1) Purified vit. B <sub>6</sub> ≡0.5 g. yeast, dry wt.           | 3, (a)                             | 1–4         | –0.7          | Animals deteriorated             |        |
|       |   |             |                      |     |     |         |               |               | (2) Unpurified yeast extracts≡0.5 g. yeast, dry wt.               | 4 { 1, (0)<br>2, (a)<br>1, (a)+(b) | 3           | 9.9           | Imperfect cures                  |        |
|       |   |             |                      |     |     |         |               |               | (3) Alcoholic extract of maize or wheat≡4 g. cereal               | 3, (a)                             | 3–4         | 11.5          | Perfect cures                    |        |
| C     | Vit. B <sub>6</sub> as a purified yeast extract≡0.5 g. yeast, dry wt. | 10          | 3                    | 0   | 6   | 1       | 2–14          | 2.6           | 12γ flavin  | 10                                 | 3–4         | 12.3          | 5 cures, 3 deteriorated,* 2 died |        |

\* These animals were afterwards cured and growth was restored when the purified vitamin B<sub>6</sub> was replaced by an unpurified yeast extract or an alcoholic extract of maize.

In the experiment detailed in Table I, the test animals were three litters of young rats which had been partially deprived of vitamin B<sub>2</sub> from birth. This was done by removing yeast from the diet of the lactating mother and, for the last week of lactation, replacing the stock diet by the usual "–B" diet, supplemented by vitamin B<sub>1</sub> as Jansen and Donath's acid clay adsorbate from rice polishings. At weaning the rats usually weighed 35–40 g. and were immediately placed in separate cages and given the experimental diet and doses of vitamins A, D, B<sub>1</sub> and either vitamin B<sub>6</sub> or flavin. 3 rats received neither component of vitamin B<sub>2</sub> and acted as controls.

Of the 10 rats which received flavin only from the time of weaning, 8 developed the florid (a) type of dermatitis, 1 showed both (a) and (b) symptoms combined, and 1 had no definite skin symptoms at the end of 14 weeks. Six of these animals, derived from two of the litters, were successfully cured by administration of vitamin B<sub>6</sub> given either as an autoclaved yeast extract, or as an alcoholic extract of maize or wheat, although the increase in weight on the combined doses tended to be subnormal, the weekly average gain being 9.9 g. in the former case and 11.5 g. in the latter. The condition of the 4 rats from the third litter was bad after the preparatory period on flavin alone, and only one

cure was successful. The others deteriorated after receiving vitamin B<sub>6</sub> as purified or unpurified yeast extract; 1 died and the other 2 were killed.

Of the 10 rats receiving vitamin B<sub>6</sub>, none showed the florid (*a*) type of skin symptoms, 6 developed the (*b*) type, one had a combination of symptoms and 3 showed no definite skin symptoms after 14 weeks on the diet. The condition of the animals receiving vitamin B<sub>6</sub> only was generally worse than that of animals receiving flavin only, even though the skin symptoms were less severe. Two of the rats in this group died shortly after the curative dose of flavin was given, 5 cured very slowly, and in 3 cases definite florid (*a*) symptoms developed within a few days after the flavin dose was given. The reason for this is not clear, but it seems as if flavin, given under these circumstances, may at first cause the pathological process responsible for the florid dermatitis to flare up temporarily, even though vitamin B<sub>6</sub> is present in the diet. The florid symptoms eventually cleared up spontaneously before the end of the experiment, but the cure was slow.

Of the 3 negative control rats which had received no vitamin B<sub>2</sub> component from the beginning of the experiment and were observed for 3 to 5 weeks, 2 showed some type (*a*) symptoms, which were rapidly cured by alcoholic extracts from maize and wheat, respectively, and 1 showed the (*b*) type. All 3 animals finally received flavin and vitamin B<sub>6</sub> and were satisfactorily cured of all symptoms and grew normally.

The results of these experiments show clearly that flavin prevents the (*b*) type and vitamin B<sub>6</sub> the (*a*) type of skin disorder. Further evidence of the effect of flavin in preventing the (*b*) type of skin lesions is recorded in the accompanying paper [Copping, 1936]. In a long series of prophylactic experiments with a modified Bourquin and Sherman diet which contains vitamin B<sub>6</sub> as an alcoholic cereal extract, the type (*a*) dermatitis was observed in only 1 rat, and the type (*b*) was shown by 37 rats. These were rapidly cured and growth was restored by the addition of flavin to the diet. Full confirmation is thus given of the conclusions of György [1934; 1935].

The above experiments also demonstrated the indispensability of both flavin and vitamin B<sub>6</sub> for maintenance as well as for healthy skin condition and suggest that in order to obtain full benefit of the one, the other must be provided in adequate amount. As in our former series [Chick *et al.*, 1935], attempts to cure by flavin or a purified concentrate of vitamin B<sub>6</sub>, respectively, were not always successful, except when alcoholic extracts of wheat or maize were used as source of vitamin B<sub>6</sub>.

#### SUMMARY.

1. In a series of prophylactic experiments with young rats maintained on diets lacking only one constituent of vitamin B<sub>2</sub>, those deprived of flavin developed the (*b*) type of skin lesions, whilst those deprived of vitamin B<sub>6</sub> developed the florid (*a*) type of dermatitis.

2. In the former case cures were observed and growth restored in 5 rats out of 10 by the administration of pure flavin. In the latter case speedy and complete cures were obtained by administration of vitamin B<sub>6</sub> as an alcoholic extract of whole maize or wheat, but cure was more irregular and imperfect when vitamin B<sub>6</sub> was given in the form of a preparation from yeast.

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