Communication to the Editor

Studies on Antivitamin B_6 Activity of Hydroxymethylpyrimidine

Abderhalden[1] discovered that 2-methyl-4-amino-5-hydroxymethylpyrimidine (I) produces a typical severe convulsion which is prevented by yeast and rice-bran. Makino *et al.*[2] observed similar effects and gave the name "toxopyrimidine" for (I) and "atoxopyrimidine" for convulsion-inhibiting substances. The latter was found to be a group of vitamin B_6 by Makino and the present writer.[3] According to Inoue[4], vitamin B_6-deficiency causes the tendency of dermatitis in man and vitamin B_6 is effective for allergic diseases such as urticaria. It is claimed that the sensitivity to allergic reactions may be connected with nutritional disturbances due to the vitamin B_6-deficiency.

(I) is produced by the hydrolysis of vitamin B_1,[5] and consequently vitamin B_1 solutions for injection always contain a slight amount of (I). It is often reported that the continuous administration of vitamin B_1 sometimes causes allergy-like side-effects.

The author is interested in the possible relationship between the toxicity of (I) and vitamin B_1 allergy and has observed the reciprocal activity between (I) and vitamin B_6. It was found through animal experiments as well as growth analysis of bacteria that (I) has an antivitamin-B_6 activity.

It became apparent that the allergy-like side-effects by the continuous administrations of high potency vitamin B_1 solutions are due to (I) which is antagonistic to vitamin B_6.

(a) **Accelerating Effect of (I) to Vitamin B_6-Deficiency Syndrome**—Albino rats weighing 40~50 g. were fed on a synthetic vitamin B_6-deficient diet and divided into two groups; the control animals were given B_6-deficient diet only and the experimental group received the same B_6-deficient diet added with (I) (5 mg. per each rat).

The growth of control rats stopped after two weeks. The experimental animals did not increase in body weight after one week and showed typical acrodynia during two weeks; there developed typical vitamin B_6-deficiency syndrome in the experimental group. Those deficiency disappeared after two more weeks when their dietary conditions were switched to a diet containing 50 γ of B_6 per each animal.

(b) **Inhibitory Effect of (I) on the Growth Action of PDN in B_6-Deficient Rats**—Weaning rats were fed on a synthetic-deficient diet for four weeks till they showed apparent weight loss. They were then devided into 4 control and 2 experimental groups. Four control groups were fed on different vitamin B_6-deficient diet supplemented with 5, 10, 25 and 50 γ per each animal of pyridoxine hydrochloride (PDN), respectively. Two experimental animals received the B_6-deficient diet containing PDN and (I); the ratio between PDN and (I) being 1:250 and 1:200, the amount of PDN being 10 and 25 γ per each rat.

Each of the control 4 groups increased in body weight in a respective average of 24.5, 32.0, 46.0, and 74.0 g. after 3 weeks of feeding. In the experiment of 10 γ PDN and 2.5 mg. (I) added to the B_6-deficient diet, the body weight of the rats increased by 12 g. after 3 weeks, while that of the corresponding control animals (PDN 10 γ) increased 32 g. In the experiment with 25 γ PDN and 5 mg. (I), the gain in weight during 3 weeks was almost the same level as that of the corresponding control, but after the 4th week, their body weight increase stopped and B_6-deficiency syndrome became apparent.

(c) **Effect of (I) on Tryptophan Metabolism in Rats**—Fifty mg. of *l*-tryptophan was administered orally into 3 following groups of rats and the amount of xanthurenic acid excreted into urine during the subsequent 24 hours was measured by the modified method of Rosen, Huff, and Perlzweig.[6]

1) B_6-Deficiency rats, fed on B_6-deficient diet for 4 weeks.

1) R. Abderhalden: Arch. ges. Physiol., **242**, 199(1939).
2) T. Makino, T. Kinoshita: Nature, **173**, 34(1954).
3) T. Makino, T, Kinoshita, Y. Aramaki, S. Shintani: *Ibid.*, **174**, 275(1954).
4) K. Inoue: Bull. Inst. Phys. Chem. Research (Tokyo), **21**, 493(1942); **22**, 305(1943).
5) A. Watanabe: J. Pharm. Soc. Japan, **59**, 133(1939).
6) F. Rosen, J. W. Huff, W. A. Perlzweig: J. Nutrition, **33**, 561(1947).

2) Recovered rats, fed on B_6-deficient diet supplemented with 25 γ B_6.
3) (I)-rats, fed on B_6-deficient diet supplemented with 25 γ and 5 mg. (I).

It was confirmed from this experiment that (I)-rats (group 3) showed a marked disturbance of tryptophan metabolism, the xanthurenic acid level being the same as that of the B_6-deficient rats (group 1), and higher than that of the recovered rats (2).

Then 50 mg. of *l*-tryptophan was orally given together with 5 mg. of (I) to rats belonging to group 2, and it was found that the simultaneous administration of (I) inhibits the tryptophan metabolism, which is reflected upon the urine xanthurenic acid excretion.

(d) **Antivitamin-B_6 Activity of (I) in the Growth of** *Saccharomyces carlsbergensis*—The antivitamin-B_6 activity of (I) which has been proved in the preceding animal experiments was tried out on the microörganisms. The inhibitory effect of (I) upon the growth of *S. carlsbergensis* (IFO 0565) on Atkin's media, in the presence and absence of vitamin B_1, was determined using turbidimetric measurements. It was found that the minimal inhibitory concentrations in the media with and without vitamin B_1 was 16 γ/cc. and 400 γ/cc., respectively.

The reversal effect of PDN against the growth inhibition caused by (I) was then determined. The concentrations of PDN necessary for the half-maximal growth was obtained from the growth curves on various amounts of (I) in the Atkin's media (supplemented with 2 γ of B_6 and 50 γ of B_1 per 200 cc.). The results were as follows:

Concentration of (I) added to the media (γ/cc.)	PDN concentration for 50% growth (γ/cc.)
50	0.0113
100	0.0251
500	0.2280

It is shown that PDN antagonizes the inhibitory effect of (I) competitively, in a certain range of concentrations on the growth of *S. carlsbergensis*.

The author wishes to thank Mr. K. Okami, Chief of the Osaka Plant, for his continued encouragement.

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March 22, 1955.