LVIII. THE EFFECT ON VITAMIN B_2 OF TREATMENT WITH NITROUS ACID.

By HARRIETTE CHICK.

From the Department of Experimental Pathology, Lister Institute, London.

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In 1918 McCollum and Simmonds reported that the dietary factor "water-soluble B" was resistant to the action of nitrous acid, the activity of the vitamin being tested by its power to restore growth in rats where increase in weight had failed on a diet deficient in this vitamin [1918]. Levene and Van der Hoeven [1926] confirmed this result. They found that the Osborne-Wakeman fraction prepared from yeast contained 90–100 % of its original "vitamin B" potency after deamination. Their tests also involved observations of the growth of rats [Levene and Van der Hoeven, 1924], but as the authors themselves admit [Levene, 1928], the time during which the rats were under observation (3–4 days) was too short for trustworthy results. McCollum and Simmonds observed the growth of their experimental animals over a period of 2 weeks.

Although the above experiments were made before the composite nature of the "water-soluble vitamin B" was recognised, it would be natural to infer that treatment with nitrous acid had not destroyed either of the two known components, vitamins B_1 and B_2, since both are necessary for growth. Peters [1924] found his antineuritic, vitamin B_1, concentrate prepared from yeast to be completely resistant to the action of nitrous acid as tested by its capacity, before and after treatment respectively, to cure polyneuritis in pigeons, but in a recent paper, Levene [1928] reports the opposite to be true of the heat-stable, antidermatitis, vitamin B_2. A vitamin B_1 concentrate, prepared by adsorption with silica gel from the Osborne-Wakeman fraction of yeast, was found to contain a small proportion of the heat-stable, vitamin B_2, factor, which could be removed by deamination. The activity of this vitamin is stated to be destroyed by treatment with nitrous acid, while the heat-labile (vitamin B_1 factor) remains intact. No description is given of the animal tests on which this statement is based.

The suggestion that the activity of vitamin B_2 might be dependent upon the presence of amino-nitrogen seemed important enough to be worth confirmation. Accordingly, the action of nitrous acid was tested on a vitamin B_2 concentrate prepared from yeast by decomposition with sulphuretted hydrogen of the precipitate formed when lead acetate is added to a dilute

acetic extract of washed brewery yeast [Chick and Roscoe, 1929]. This concentrate contained about 6 % of the dry weight, 3 % of the nitrogen and about one-half the vitamin B_2 of the original yeast. The daily dose required to maintain normal growth (weekly increase in weight of 11–14 g.) in young rats on a diet deprived only of vitamin B_2 was 0·03 g. (dry weight, less ash), and was contained in the equivalent of 0·5 g. of the original yeast. Vitamin B_1 was also present.

Deamination was carried out as follows, using the method described by Peters [1924]. To 50 cc. of the preparation (equivalent to 50 g. original dried yeast), which was already acid (p_H *ca.* 3·0), 50 cc. distilled water, 5 cc. of a 30 % solution of sodium nitrite and 10 cc. of 10 % sulphuric acid were added. The mixture was shaken by hand for 5 minutes, left to stand overnight, heated next day on a water-bath, and after all foaming had ceased, allowed to boil for 10 minutes. Estimations by Van Slyke's method (15 mins. shaking) gave 0·091 % amino-nitrogen (*i.e.* 0·091 g. per 100 g. of the original yeast) in the material before treatment, and this was reduced to about one-sixth, viz. to 0·016 % amino-nitrogen after treatment.

Table I. *Influence of deamination upon the vitamin B_2 activity of a preparation (XII_3C) obtained from brewer's yeast, daily dose* 0·5 *cc., equivalent to* 0·5 *g. dried yeast.*

The rats received diet P_2L deprived of B vitamins supplemented by daily doses of cod-liver oil (0·05–0·1 g.), to provide vitamins A and D, and of Peters's antineuritic concentrate (0·1 cc. equivalent of 0·6 g. yeast), to provide vitamin B_1.

Material	Nitrogen in preparation (reckoned per 100 g. of original yeast)		Litter No.	Rat No.	Body wt g.	Increase in body weight in successive weeks g.	Average	Mean
	Total	Amino						
Before treatment with nitrous acid	0·24	0·091	1264	451 ♀	36	15, 12, 13	13·3	14·0
			1285	452 ♂	41	16, 11, 15	14	
			„	455 ♀	35	18, 14, 12	14·7	
After treatment with nitrous acid	0·13	0·016	1264	450 ♀	40	15, 14, 12	13·7	13·8
			1285	453 ♂	43	16, 17, 11	14·7	
			„	454 ♀	37	12, 13, 14	13	

No diminution in the vitamin B_2 potency of the preparation could be detected. The results of the tests, using our method of vitamin B_2 assay [Chick and Roscoe, 1928], are set out in the accompanying Table I. Six young rats (35–43 g. weight) were divided into two groups, similar in respect of litter and sex, and maintained on a basal diet deprived of B vitamins with the addition of a daily dose (0·1 cc., equivalent to 0·6 g. yeast) of Peters's antineuritic concentrate, to provide vitamin B_1. One group of rats received the vitamin B_2 concentrate before treatment with nitrous acid (daily dose equivalent to 0·5 g. original dried yeast) and the second an equal dose of the material after deamination. Growth of both groups was observed for 3 weeks. The rats in the first group showed an average weekly increase in weight of 14·0 g. and those in the second of 13·8 g., proving that no destruction of vitamin B_2 had taken place during treatment with nitrous acid.

SUMMARY.

Levene's observation that the activity of vitamin B_2 is destroyed by the action of nitrous acid is not confirmed. A vitamin B_2 preparation from yeast was found to possess equal power after and before treatment to induce growth in young rats on a diet deprived of this vitamin.

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