

LXXXII. IMPROVED YIELDS OF VITAMIN B₁.

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THE yields previously reported [Kinnersley *et al.*, 1933] have now been improved and some changes introduced. By using a special yeast supplied by the Distillers Company Ltd., about four times the amount of crystals are obtained from a given amount of yeast; 60 mg. pure hydrochloride may be expected from 50 kg. moist yeast (10 kg. as dry). It is an improvement to adsorb on charcoal at p_H 6.5 as the vitamin seems less liable to damage at this p_H value. The special yeast does not give a clear filtrate on centrifuging after the initial boiling. This can be clarified by the use of more lead acetate, at least double the amount previously recommended. It is apt to filter rather slowly at the baryta stage. The phosphotungstate stage works with precision; we notice that others have confirmed its value [Williams *et al.*, 1934]; the formaldehyde-azo-test is here especially useful and we place increasing reliance upon it [Kinnersley and Peters, 1934].

In the final stages of crystallisation, after removing the bulk of the crystals with acid alcohol (HCl, p_H 3), containing about 10 mg. of crystals in each ml., the remaining vitamin may be obtained as crystals by concentrating the filtrates to about half the volume and adding light petroleum (B.P. 40–60°) to the hot solution to faint cloudiness and cooling. Some further 15 % yield may thus be obtained.

As previously recorded (and as confirmed by Williams *et al.* [1934]), loss occurs at the gold stage, presumably owing to oxidation. We have tried to eliminate this by addition of cyanide but have obtained no certain improvement; in some experiments cyanide decreased the yield. Exp. 1 is an example of several experiments of this type.

Exp. 1. *The precipitation of the activity by gold chloride in presence of KCN.* The alcohol was removed *in vacuo* from a volume of the 99 % alcohol solution equivalent to $\frac{1}{2}$ cwt. yeast (20,000 doses); 15 ml. of water were added and about 3 ml. of 10 % gold chloride and 1 ml. 10 % KCN alternately and in small and proportionate volumes at a time; the 30 ml. to be centrifuged were meanwhile cooled in ice. After a few minutes the precipitate was centrifuged off and without being washed was suspended in 15 ml. *N*/200 HCl, and H₂S was passed through for a period of 1 to 2 hours. The sulphide precipitate was filtered under micro-conditions and the filtrate concentrated in a small evaporating basin on the water-bath with 1 drop of 10 % BaCl₂ to about 0.5 ml. or less. During the last stages, the side of the basin was washed with alcohol, with which solvent the residue was finally removed into a small 5 ml. tared tube. In this tube most of the water was removed by heating and shaking in the bath—any inactive precipitates were centrifuged off and the solution was allowed to crystallise in the cold chamber for 24 hours or longer.

Equal amounts representing 25 kg. yeast were worked up with and without KCN. The yields were, with KCN: 21.3, 25.6, 30.5; without KCN: 35 mg.

The gold stage seems indispensable for crystallisation; loss is best avoided by minimum times of precipitation (5 mins.) and the use of cooling. We have been unable to convince ourselves that the passage of nitrogen is definitely helpful.

Table I gives the present yields. It must be realised that there are also proportionately increased yields of cruder vitamin B₁ present in the 50 % alcoholic extracts of charcoal which we find very useful in other work.

Table I. *Vitamin B₁ yields from special baker's yeast (moist).*

The amounts of vitamin hydrochloride present unless otherwise stated are judged by colour test in which 2γ=1 dose vitamin.

Date	Batch	Amount kg.	Pigeon doses		Pure crystals mg.	mg./50 kg.
			99 % alcohol stage	Phosphotungstate stage (p _H 5)		
6. ii. 33	87	25	20,000	30,000	34.6	73.2
6. ii. 33	89	200	150,000	150,000		
7. ii. 33	89	100	80,000	145,000 (Bird)	222.0	55.5
			90,000 (Bird)	—	130	65
4. iii. 34	33	100	100,000	90,000	131.5	65.7
8. iii. 34	47	200	220,000			
			230,000 (Bird)	140,000	210	52
					Average	62.3

SUMMARY.

Improvements in the yield of pure crystalline vitamin B₁ hydrochloride are described.

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REFERENCES.

- Kinnersley, O'Brien and Peters (1933). *Biochem. J.* **27**, 232.
 — and Peters (1934). *Biochem. J.* **28**, 667.
 Williams, Waterman, Keresztesy (1934). *J. Biol. Chem.* **56**, 1187.

Note. In the paper by Kinnersley and Peters [1934], on page 668, line 28, delete "of p_H 5".