Thiamine, Pyrimidine and Thiazole as Bios Factors

By Alfred S. Schultz, Lawrence Atkin and Charles N. Frey

In view of our discovery of the fermentation stimulating effect of thiamine,[1,2] studies were instituted relative to thiamine as a bios factor. Thiamine is a growth-active substance for various yeasts. Amongst strains of *Sacch. cerevisiae* the response to thiamine as a bios factor differs.

Table I

Total volume in each case 30 ml. seeded with 1 mg. of moist yeast and rocked at 30° for 24 hours. Crop × 4.54 gives mg. of moist yeast. Supplements: inositol [I] 1 mg., β-alamine [IIA] 0.005 mg., bios II B 0.13 mg., thiamine 0.01 mg., thiazole 0.01 mg., aminopyrimidine 0.01 mg., bios VI 1 cc. of a concentrate.

Ingredients of bios test	Crop Type A	Type B
Sugar, salts, buffer, I, IIA, IIB	30	220
Sugar plus thiamine	120	120
Sugar plus thiamine and bios VI	220	220
Sugar plus thiazole	70	220
Sugar plus aminopyrimidine	80	200
Sugar plus aminopyrimidine + thiazole	120	150

Thus type A is stimulated by thiamine and type B is inhibited by it. This is true when the growth medium contains bios I (inositol), bios IIA (beta-alanine) and bios IIB. As may be seen it is possible to add another factor (bios VI, we have named it), which has not been freed of the other bioses but which may be found in many places. This factor further stimulates type A while removing the inhibition on type B. Examples of type A cultures are Luft II, and Rasse XII. Type B cultures are represented by *Sacch. cerevisiae* Toronto and Spc. 152.

Included in the table are results obtained with components of thiamine. "Thiazole" in the table is 4-methyl-5-beta-hydroxyethylthiazole and "aminopyrimidine" is 2-methyl-5-ethoxymethyl-6-aminopyrimidine. Thus type A yeast is partly stimulated by either fraction and completely activated by a combination. Type B yeast is not affected by the thiazole and is only slightly inhibited by the aminopyrimidine. The combination, however, inhibits type B to the same extent as thiamine.

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(1) Schultz, Atkin and Frey, This Journal, **59**, 948 (1937).
(2) *Idem.*, p. 2457.

On the Calculation of the Dissociation Constants of Hypohalogenous Acids from Kinetic Data

By Eugene A. Shilov

The author recently has determined the dissociation constant of hypobromous acid by electrochemical titration with glass electrode and found it to be 2.06×10^{-9} at 20°.

This value is in disagreement with that obtained by Chapin[1] by means of the kinetical method, namely, 2.5×10^{-8}, later adopted by Prutton and Maron[2] in the calculations of their measurements of the kinetics of hypobromite decomposition.

The kinetical method used first by Gallart[3] is based on the determination of the pH at which the velocity of a hypohalite decomposition is maximal. Under assumption that the kinetical equation of the reaction has the form

$$-\frac{d[OX^-]}{dt} = k[HOX]^2[OX^-] \qquad (1)$$

$(p\mathrm{H})_{max.}$ must correspond to one-third neutralization of the hypohalogenous acid present. From this relation in combination with the equation $K = [H^+][OX^-]/[HOX]$ it follows

$$\log K = -(p\mathrm{H})_{max.} - \log 2$$

The method involves two sources of errors: (1) experimental errors of the determination of $(p\mathrm{H})_{max.}$; and (2) the inexactitude of the equation (1).

On examining the decomposition curves of hypochlorite in the article of Chapin[1] the probable error of determination of $(p\mathrm{H})_{max.}$ on the curve appears to be 0.2–0.3 pH. The total experimental error of the determination is apparently much larger, as the values of different authors vary very much between themselves. Gallart[3] and Chapin[1] have obtained in the decomposition of hypochlorite $(p\mathrm{H})_{max.} = 6.7$ and hence $K_{HOCl} = 10^{-7}$, but Markuse[4] determined $(p\mathrm{H})_{max.}$ as >7, and hence $K_{HOCl} < 5 \times 10^{-8}$.[5]

It seems that the control of pH in the investigations of Chapin and particularly of Markuse did

(1) Chapin, This Journal, **56**, 2211 (1934).
(2) Prutton and Maron, *ibid.*, **57**, 1653 (1935).
(3) Gallart, *Anales Soc. españ. fis. quím.*, **31**, 422 (1933), cited from Chapin.[1]
(4) Markuse, *Reconstruction Text. Ind.* (Russ.), **5**, 43 (1935).
(5) Here may be noted also the earlier investigation of Giordani [*Gazz. chim. ital.*, **54**, 844 (1924)], who calculated the constant of hydrolysis of hypochlorite from the measurements of the kinetics of sodium hypochlorite decomposition in very alkaline solutions (pH > 13). The value obtained by Giordani ($K_{Hydr.} = 1.12 \times 10^{-6}$ at 30°, $K_{HOCl} = 1.5 \times 10^{-8}$) is near to modern values, but perhaps only accidentally, as the method of Giordani is not exact enough.