ical groups characteristic of the amino acids of proteins can be applied directly to the quantitative determinations of the amino acids of feedingstuffs with at least a fair degree of accuracy.

2. The results which we have obtained for the quantitative determination of amino acids in feedingstuffs, on the whole, do not agree well with those recently published by Nollau. In some determinations the results from the two sources are quite satisfactory, but in many cases the agreement is far from satisfactory. The lack of concordant results is probably due in the main to differences in the details of procedure in the experimental work.

3. The results reported in this paper show that there are pronounced variations in the free and combined amino-acid content of the common feedingstuffs expressed in percentage of the total nitrogen. There are also wide and marked variations in the distribution of the nitrogen of the free and combined amino acids in the feedingstuffs, expressed in percentage of the feedingstuff.

4. It seems probable that the high results for humin nitrogen obtained in the direct analysis of feedingstuffs by the Van Slyke method are due, in part, to the presence of soluble carbohydrates during the hydrolysis of the proteins. It also seems probable that the high humin nitrogen which is obtained in the analysis of feedingstuffs may be due, in part, to the presence of cellulose, which mechanically prevents the complete hydrolysis of the proteins.

5. The high results for humin nitrogen constitute a source of error in the direct application of the Van Slyke method to the determination of the free and combined amino acids and amides of feedingstuffs.

URBANA, ILL.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF COLUMBIA UNIVERSITY AND THE HARRIMAN RESEARCH LABORATORY, NO. 257.]

# THE EFFECT OF SODIUM CHLORIDE UPON THE ACTION OF INVERTASE.

BY H. A. FALES AND J. M. NELSON.

Received September 15, 1915.

The object of the work described in this paper is the study of the influence of sodium chloride upon the hydrolysis of cane sugar by invertase. The great susceptibility of the action of invertase to small amounts of acids and alkalies has been observed by Kjeldahl,[1] O'Sullivan and Tompson,[2] and others.

Sörensen[3] made an important advance when he showed that the ac-

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[1] *Meddelelser fra Carlsberg Laboratorit*, 1, 337 (1881).

[2] *J. Chem. Soc.*, 57, 835 (1890).

[3] *Biochem. Ztg.*, 21, 131–304 (1909).

tivity of invertase is a function of the concentration of hydrogen ion rather than of the absolute amount of acid present. Furthermore, for any given concentration of hydrogen ion, the activity of the invertase is independent of the nature of the anion furnished by the acids that he tried, namely, hydrochloric, sulfuric, phosphoric and citric. He also measured the particular concentration of hydrogen ion at which invertase exhibits its maximum activity, and found it to be in the neighborhood of $10^{-4.5}$ mols hydrogen ion per liter at a temperature of 52.5°. We are greatly indebted to Sörensen for working out in detail the methods for the measurement of the concentration of hydrogen ion in enzymatic reactions in general. The results of Michaelis and Davidsohn[1] and of the present work confirm Sörensen's observations as to the optimum zone of invertase activity. One may, therefore, accept with reasonable assurance that the relationship existing between the concentration of hydrogen ion and the activity of invertase has been definitely determined.

As regards the effect of salts upon the activity of invertase, it seems that the most extensive work with this purpose in view is that of Cole,[2] but his results do not admit of any reliable conclusions because he did not record the concentration of hydrogen ion in his respective experiments. As he himself says:

> "I have tried the effect of mixtures of HCl and chlorides on the action of invertin but the results have been very unexpected and difficult to interpret."

Michaelis and Davidsohn[3] carried out one experiment with $M/710$ potassium chloride, but consider that the concentration of the potassium chloride is too small to exert any disturbing influence. They also tried an experiment with $M/225$ potassium chloride, and one with $M/150$ potassium chloride but they do not discuss their results with reference to the salt effect.

In view of the negative results of Cole and because of the recognized importance of the influence of salts in hydrolytic cleavage reactions in general,[4] the authors of this paper deemed it advisable to start with the study of the effect of sodium chloride upon the activity of invertase in the hydrolysis of cane sugar. In the elaboration of the experimental detail, they have been guided by two main considerations: the one of theoretical interest to see if further insight could not be gained into the mechanism of the inversion of cane sugar; the other of practical value to determine whether the use of so-called "buffers" (mixtures of acids and salts) which many investigators employ to secure their desired concen-

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[1] *Biochem. Ztg.*, **35**, 386 (1911).

[2] *J. Physiol.*, **30**, 281 (1904).

[3] *Loc. cit.*

[4] Vide Spohr, *Z. physik. Chem.*, **2**, 194 (1888); Arrhenius, *Ibid.*, **4**, 226 (1889); Euler, *Ibid.*, **32**, 348 (1900).

tration of hydrogen ion, gives results which are comparable with those obtained for the same concentration of hydrogen ion secured by the use of acids or alkalies alone.

The general plan of procedure in this work has been to measure the activity of invertase at various concentrations of hydrogen ion ranging from $10^{-1.4}$ to $10^{-6.6}$, then to measure the activity over the same range of hydrogen ion concentrations in the presence of 0.1, 0.5 and 2 molar sodium chloride.

The relationship between the activity of invertase and hydrogen ion concentration has been found to be briefly as follows for the conditions given: total volume of solution, 100 cc.; amount of cane sugar, 0.500 g.; amount of invertase, 10 cc. of working solution of invertase;[1] concentration of hydrochloric acid, enough to give the desired concentration of hydrogen ion; temperature 37° ± 0.01°. For details, see experiments Nos. 2, 7, 11 and 17.

| Value of $p_H^+$ | 1.4 | 3.1 | 3.3 | 4.5 | 6.0 | 6.3 | 6.5 |
|---|---|---|---|---|---|---|---|
| Activity | None | 75 | 85 | 94 | 51 | 33 | 26 |

The term $p_H^+$ is Sörensen's symbol for expressing the concentration of the hydrogen ion. The values are the negative exponents to which the number ten must be raised in order to equal the given concentration. Thus, $p_H^+ = 4.5$ means that the concentration of hydrogen ion is equal to $10^{-4.5} = 0.00003$ mol hydrogen per liter. Since the values of $p_H^+$ represent negative exponents, it follows that the smaller the numerical value of $p_H^+$ the larger the concentration of hydrogen ion and *vice versa;* for 0.1 *M* hydrochloric acid $p_H^+ = 1.04$, while for 0.1 *M* sodium hydroxide $p_H^+ = 13.06$. The authors have found it very convenient to designate the symbol $p_H^+$ by the term "index" and it is employed in this sense throughout this article.

Armstrong and Armstrong[2] strongly object to indicating the concentration of hydrogen ion in this way, but it would seem that they are not fully familiar with the scheme as is evident from the following quotation from their article:

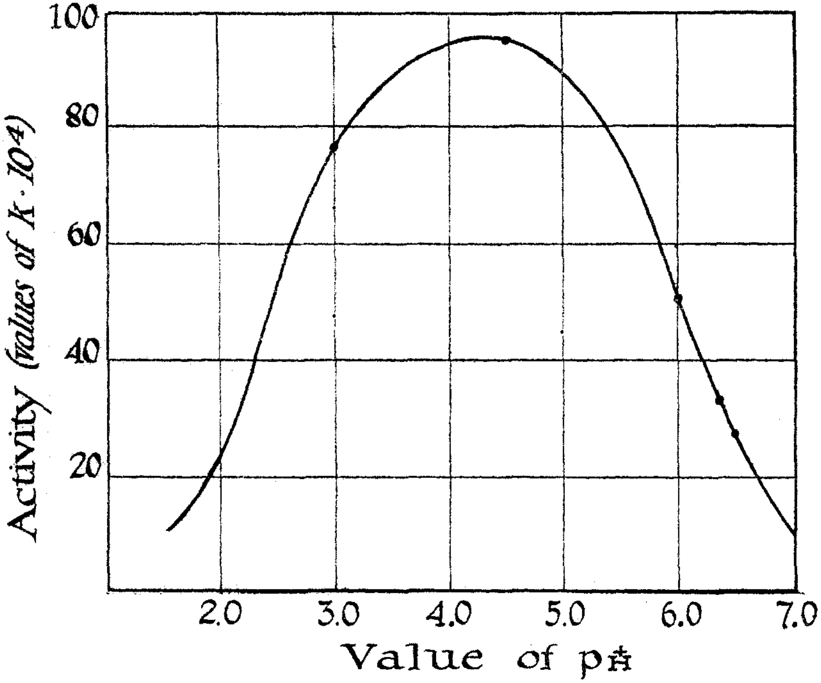
> "It is difficult enough for nonmathematical readers to appreciate values stated in terms of the expression $x \times 10^{-7}$ or $10^{-y}$ but it is still more difficult for them to follow the method adopted by Sörensen, the first to introduce order, and one of the chief workers in this field, who uses the indices alone (the $y$ values) as the exponents of the hydrogen-ion concentration, so that values below 7 indicate alkalinity and those above 7 acidity."

[1] Prepared each time as wanted by diluting 10 cc. of a stock solution of invertase to 100 cc. and then taking 10 cc. portions from this 100 cc. The stock solution was made from invertase prepared by Nelson and Born (THIS JOURNAL, **36**, 393 (1914)) and contained 7 g. invertase per liter. It showed no decrease in activity after being kept for one year in the ice box.

[2] *Proc. Roy. Soc.*, (*B*) **86**, 576 (1913).

**Activity.**—The values in the activity column are the values of the velocity coefficients × $10^4$ calculated from the monomolecular equation $k = \frac{1}{t} \log \frac{a}{a - x}$ (A), where $t$ is the time in minutes, and the logarithms are those to the base ten. It is to be pointed out that the values of the velocity coefficients calculated from this equation differ somewhat from those calculated from the equation $k = \frac{1}{t_2 - t_1} \log \frac{a - x_1}{a - x_2}$ (B). The use of Equation A seems to give smoother results. It is, therefore, necessary in comparing values obtained by different workers to see that they are evaluated by means of the same equation, as is evidenced by taking

Figure No 1



| Mins. | % Inversion. | (A). | (B). |
|---|---|---|---|
| 5 | 3.1 | 0.0027 | 0.0031 |
| 15 | 9.8 | 0.0030 | 0.0032 |
| 30 | 19.2 | 0.0031 | 0.0032 |
| 57 | 33.6 | 0.0031 | 0.0027 |
| 90 | 45.8 | 0.0030 | 0.0039 |
| 120 | 58.5 | 0.0032 | 0.0035 |
| 150 | 67.4 | 0.0032 | 0.0033 |
| 182 | 74.5 | 0.0033 | 0.0036 |
| 210 | 79.8 | 0.0033 | 0.0037 |
| 240 | 84.4 | 0.0034 | 0.0030 |
| 270 | 87.3 | 0.0033 | 0.0026 |
| 381 | 93.5 | 0.0031 | 0.0025 |
| 430 | 95.1 | 0.0032[1] | 0.0016 |
| 508 | 96.3 | 0.0028[1] | 0.0007 |

[1] Calculated by the authors of this paper.

one of O'Sullivan and Tompson's experiments,[1] the results of which have been calculated by Hudson[2] according to (A) and by Sörensen[1] according to (B).

The above illustration has been cited because of the importance that the velocity coefficients have played in the discussions as to whether the inversion of cane sugar by invertase can be regarded strictly as a monomolecular reaction or not.

**Effect of Salt.**—In adjusting the acidity of the solutions for the tests at an index of 1.5, it was found that when the same amounts of hydrochloric acid were made up to the same volume with water and with solutions of sodium chloride so that the resulting concentration of sodium chloride was respectively 0.1, 0.5 and 2.0 *M*, the resulting indices of the solutions, as measured by the electromotive force method (described subsequently in this article) were in each case different, and in such wise that the greater the concentration of sodium chloride, the less the value of the index; or in other words, the greater the concentration of hydrogen ion.

Thus the following results were obtained, when in the final volume of the solution the concentration of the hydrochloric acid was 0.045 *M*. and the concentration of sodium chloride as given:

| | No. salt. | 0.1 *M* NaCl. | 0.5 *M* NaCl. | 2.0 *M* NaCl. |
|---|---|---|---|---|
| E. m. f. (volts) at 25° | 0.4185 | 0.4160 | 0.4100 | 0.3936 |
| Value of $p_{H^+}$ | 1.41 | 1.37 | 1.27 | 1.00 |
| Conc. of hydrogen ion | 0.039 | 0.043 | 0.054 | 0.100 |
| % increase of $H^+$ | .. | 10.3 | 38.5 | 156.0 |

This increase of hydrogen ion concentration seems also to be in line with the increase of the velocity coefficients obtained in the hydrolysis of cane sugar under the same conditions as regards concentration of hydrochloric acid and concentration of sodium chloride (see Experiments Nos. 1, 3 and 5) as is shown herewith:

| | No. salt. | 0.1 *M* NaCl. | 0.5 *M* NaCl. | 2.0 *M* NaCl. |
|---|---|---|---|---|
| Vel. coeff. at 37°. Conc. HCl 0.045 *M* | 0.00073 | .... | 0.00093 | 0.00176 |
| % increase of vel. coeff | ... | .... | 27.4 | 141.0 |

Loomis and Acree[3] observed that the addition of potassium chloride to acetic acid solutions slightly increases the dissociation of the acetic acid, although the validity of their results has been questioned by McBain and Coleman.[4] B. von Szyzkowski[5] cites the fact, which the authors have also noticed, that methyl orange behaves as if the concentration of

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[1] *Loc. cit.*

[2] THIS JOURNAL, **30,** 1160 (1908).

[3] *Am. Chem. J.*, **46,** 621 (1911).

[4] *J. Chem. Soc.*, **105,** 1517 (1914).

[5] *Z. physik. Chem.*, **78,** 426 (1912).

hydrogen ion is increased when sodium chloride is added to a very dilute solution of hydrochloric acid. Poma[1] states that the presence of sodium chloride as well as the chlorides of other alkali metals increases the concentration of hydrogen ion of hydrochloric acid solutions.

Apart from whatever theoretical importance the above observation may have, it illustrates the necessity of actually measuring the concentration of hydrogen ion in all such experiments rather than calculating it from the molarity of the acid used and the concentration of the salt added.

In the experiments which follow (1–17) 10 cc. of cane sugar solution, containing 0.500 g. of cane sugar were used in every instance, together with the other additions as indicated. The temperature was 37 ± 0.01°.

**Effect at $p_{H^+} = 1.40$.**—The results of the measurements of the activity of invertase at an index of 1.40 and less, show that invertase has no inverting action, irrespective of whether there is no sodium chloride, 0.1, 0.5 or 2.0 *M* sodium chloride present. (See Expts. 1 to 6 inclusive.)

TABLE I.

**Expts. 1-6**; 9 cc. of 0.5 *M* HCl added in every case, other additions as noted.

**No. 1**: 81 cc. water; $p_{H^+}$ = 1.41 (e. m. f. 25°).

| Minutes | 180 | 210 | 225 | 255 | 285 |
|---|---|---|---|---|---|
| Mg. cane sugar inverted | 12.4 | 14.8 | 16.0 | 17.6 | 19.4 |
| K × $10^4$ (acid alone) | 6.9 | 7.3 | 7.4 | 7.4 | 7.5 |

**No. 2**: +71 cc. water; +10 cc. invertase working solution containing 0.007 g. invertase; $p_{H^+}$ = 1.42, 1.43 (e. m. f. 25°).

| Minutes | 180 | 210 | 225 | 255 | 285 |
|---|---|---|---|---|---|
| Mg. cane sugar inverted | 12.4 | 14.8 | 16.0 | 17.6 | 19.4 |
| K × $10^4$ | No inversion by invertase. | | | | |

**No. 3**: +31 cc. water; +50 cc. 1.0 *M* NaCl; $p_{H^+}$ = 1.27, 1.27 (e. m. f. 25°).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | .. | 2.6 | 4.7 | 6.8 | 8.8 | 11.5 |
| K × $10^4$ (acid + NaCl) | .. | 7.7 | 9.5 | 10.6 | 9.3 | 9.5 |

**No. 4**: +21 cc. water; +50 cc. 1.0 *M* NaCl; +10 cc. invertase working solution containing 0.007 g. invertase; $p_{H^+}$ = 1.27, 1.27 (e. m. f. 25°).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | .. | 2.6 | 4.7 | 6.8 | 8.8 | 11.5 |
| K × $10^4$ | No inversion by invertase. | | | | | |

**No. 5**: +31 cc. water; +50 cc. 4.0 *M* NaCl; = $p_{H^+}$ = 1.00, 1.00 (e. m. f. 25°).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 2.0 | 5.3 | 8.5 | 11.7 | 15.1 | 19.1 |
| K × $10^4$ (acid + NaCl) | 11.8 | 16.2 | 18.0 | 19.3 | 17.3 | 17.4 |

**No. 6**: +21 cc. water; +50 cc. 4.0 *M* NaCl; +10 cc. invertase working solution containing 0.007 g. invertase; $p_{H^+}$ = 1.00, 1.00 (e. m. f. 25°).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 2.0 | 5.3 | 8.5 | 11.7 | 15.1 | 19.1 |
| K × $10^4$ | No inversion by invertase. | | | | | |

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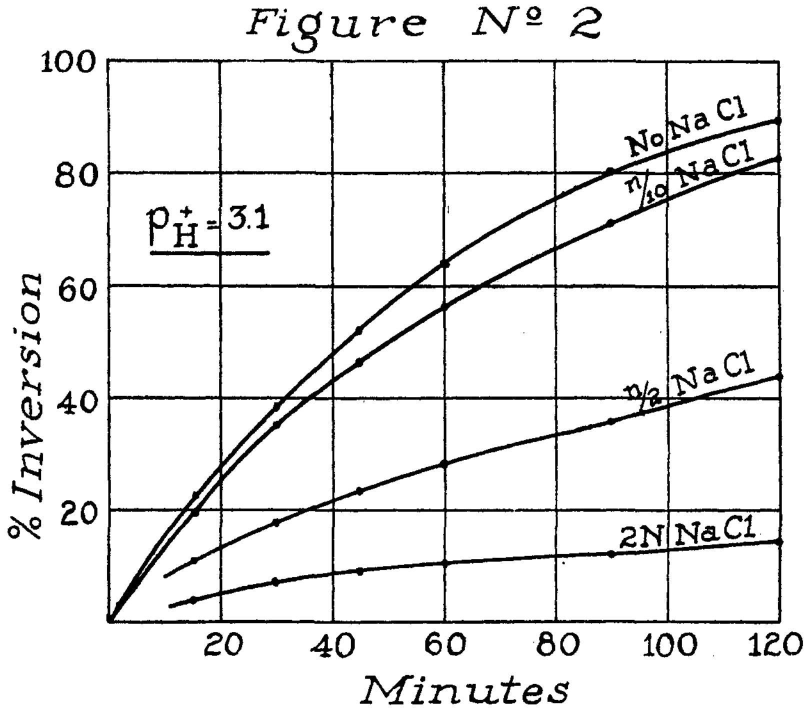
[1] *Z. physik. Chem.*, 88, 641 (1914).

**Effect at $p_H^+ = 3.1$.**—The results at an index of about 3.1 show a progressively retarding effect of the sodium chloride as evidenced by the following values, which are represented graphically in Fig. 2. For details, see Expts. 7, 8, 9 and 10.



PERCENTAGE INVERSION AT TIME GIVEN.

| Mins. | No salt. $p_H^+ = 3.1$. | 0.1 *M* NaCl. $p_H^+ = 3.2$. | 0.5 *M* NaCl. $p_H^+ = 3.1$. | 2.0 *M* NaCl. $p_H^+ = 3.0$. |
|---|---|---|---|---|
| 15 | 22.4 | 19.8 | 11.8 | 4.0 |
| 30 | 39.6 | 34.0 | 18.4 | 8.0 |
| 45 | 52.8 | 46.0 | 23.0 | 8.2 |
| 60 | 64.6 | 57.4 | 28.4 | 10.4 |
| 90 | 80.6 | 70.1 | 35.0 | 12.2 |
| 120 | 89.0 | 82.6 | 44.0 | 13.8 |

TABLE II.

**Expts. 7-10, incl.**; 10 cc. cane sugar; 0.7 cc. 0.1 *M* HCl (except No. 10 with 0.5 cc.); 10 cc. invertase working soln. contg. 0.007 g. invertase; other additions as noted.

No. 7: +80 cc. water; $p_H^+ = 3.2, 3.2$ (e. m. f. 25°); $p_H^+ = 3.1, 3.2$ (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 11.2 | 19.8 | 26.4 | 32.3 | 40.3 | 44.5 |
| $K \times 10^4$ | 73 | 73 | 73 | 75 | 79 | 80 |

TABLE II (*continued*).

**No. 8:** +70 cc. water; +10 cc. 1.0 *M* NaCl; $p_{H^+}$ = 3.22, 3.22 (e. m. f. 25°); $p_{H^+}$ 3.3, 3.2 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 9.9 | 17.0 | 23.0 | 28.7 | 35.5 | 41.3 |
| $K \times 10^4$ | 64 | 60 | 60 | 62 | 60 | 63 |

**No. 9:** +30 cc. water; +50 cc. 1.0 *M* NaCl; $p_{H^+}$ = 3.0, 3.0 (e. m. f. 25°); $p_{H^+}$ = 3.1, 3.2 (by methyl orange).

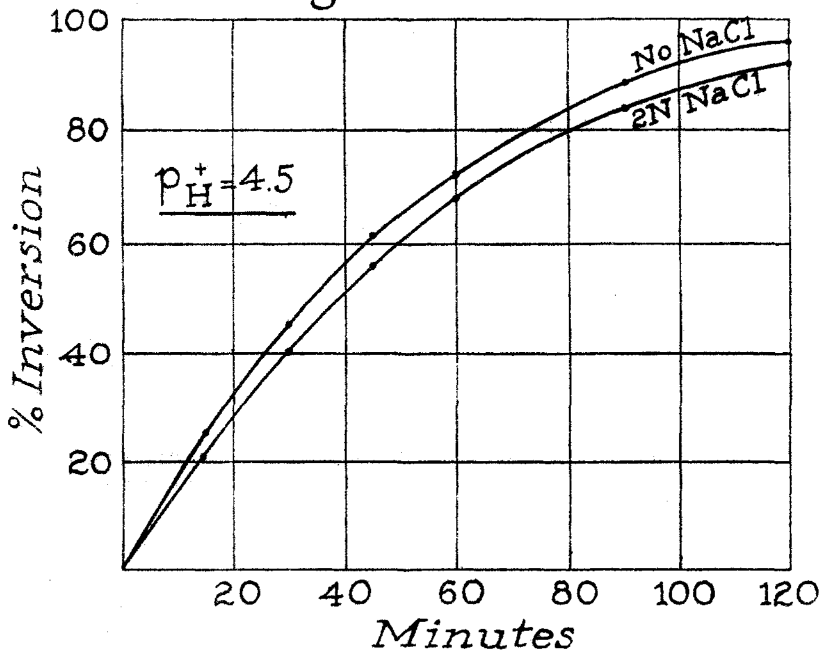
| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 5.9 | 9.2 | 11.5 | 14.2 | 17.5 | 22.2 |
| $K \times 10^4$ | 36 | 29 | 25 | 24 | 22 | 21 |

**No. 10:** +30 cc. water; +50 cc. 4.0 *M* NaCl; $p_{H^+}$ = 3.0, 3.0 (e. m. f. 25°); $p_{H^+}$ = 2.7, 2.7 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 2.0 | 4.0 | 4.1 | 5.2 | 6.1 | 6.9 |
| $K \times 10^4$ | 12 | 12 | 8 | 8 | 6 | 5 |

**Effect at $p_{H^+}$ = 4.5.**—The investigation of the salt effect at an index of 4.5 reveals the rather surprising result that in this region, sodium chloride, at least below the concentration of 2 molar, has very little effect upon the activity of invertase. The graphs (see Fig. 3) all lie so close together that only the one for no salt and the one for 2 molar sodium chloride have been drawn. The other two graphs are practically con-

Figure № 3



tained in the space between these two. For details, see Expts. 11, 12, 13 and 14.

PERCENTAGE OF INVERSION AT TIME GIVEN.

| Mins. | No salt. $p_{\mathrm{H}}+ = 4.6$. | 0.1 *M* NaCl. $p_{\mathrm{H}}+ = 4.4$. | 0.5 *M* NaCl. $p_{\mathrm{H}}+ = 4.6$. | 2.0 *M* NaCl. $p_{\mathrm{H}}+ 4.5$. |
|---|---|---|---|---|
| 15 | 25.8 | 26.0 | 25.8 | 20.8 |
| 30 | 45.2 | 45.1 | 44.2 | 40.0 |
| 45 | 62.6 | 60.0 | 61.0 | 57.6 |
| 60 | 72.8 | 72.6 | 72.6 | 68.2 |
| 90 | 87.2 | 86.6 | 88.8 | 84.4 |
| 120 | 94.6 | 96.6 | 95.4 | 92.0 |

TABLE III.

**Expts. 11-14, incl.**; 10 cc. cane sugar; +10 cc. invertase working soln. contg. 0.007 g. invertase; + other additions as noted.

No. 11: +80 cc. water; +1.1 cc. 0.01 *M* HCl; $p_{\mathrm{H}^+}$ = 4.6, 4.6 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 12.9 | 22.6 | 28.7 | 36.4 | 43.6 | 47.3 |
| $K \times 10^4$ | 86 | 87 | 95 | 94 | 99 | 106 |

**No. 12**: +70 cc. water; +10 cc. 1.0 *M* NaCl; +1 cc. 0.01 *M* HCl; $p_{\mathrm{H}^+}$ = 4.5, 4.5 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 12.9 | 22.6 | 30.0 | 36.3 | 43.3 | 48.3 |
| $K \times 10^4$ | 86 | 87 | 89 | 94 | 97 | 122 |

**No. 13**: +30 cc. water; +50 cc. 1.0 *M* NaCl; +0.7 cc. 0.1 *M* HCl; $p_{\mathrm{H}^+}$ = 4.6, 4.5 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 12.9 | 22.1 | 30.5 | 36.3 | 44.4 | 47.7 |
| $K \times 10^4$ | 86 | 84 | 91 | 94 | 106 | 111 |

**No. 14**: +30 cc. water; +50 cc. 4.0 *M* NaCl; +0.4 cc. 0.01 *M* HCl; $p_{\mathrm{H}^+}$ = 4.1, 4.3 (e. m. f. 25°); $p_{\mathrm{H}^+}$ = 4.4, 4.3 (by methyl orange).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 10.0 | 20.0 | 28.4 | 32.8 | 42.8 | 46.0 |
| $K \times 10^4$ | 65 | 74 | 81 | 77 | 93 | 91 |

**Effect at $p_{\mathrm{H}^+}$ = 6.5.**—In the region having an index of about 6.5, the sodium chloride causes a decrease in the activity of the invertase much the same way that it does in the region having an index of 3.1. For details, see Expts. 15, 16 and 17.

PERCENTAGE INVERSION AT TIME GIVEN.

| Mins. | No salt. $p_{\mathrm{H}}+ = 6.5$. | 0.1 *M* NaCl. $p_{\mathrm{H}}+ = 6.4$. | 0.5 *M* NaCl. $p_{\mathrm{H}} += 6.5$. |
|---|---|---|---|
| 15 | 11.6 | 9.0 | 4.8 |
| 30 | 19.2 | 15.2 | 8.6 |
| 45 | 24.0 | 19.8 | 12.6 |
| 60 | 29.6 | 25.2 | 16.4 |
| 90 | 42.4 | 33.6 | 21.6 |
| 120 | 51.0 | 40.8 | 26.8 |

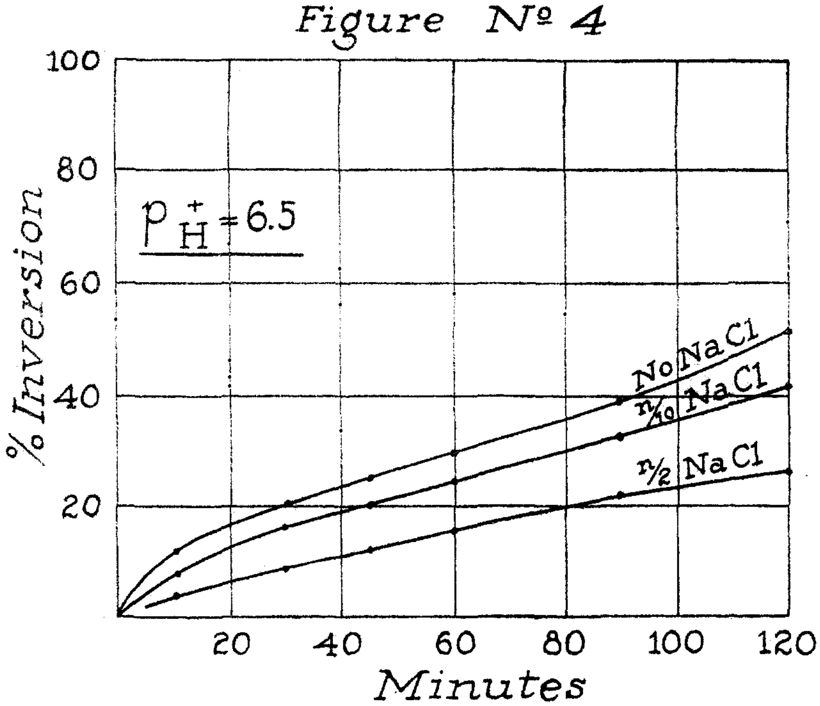


Table IV.

**Expts. 15-17, incl.**; 10 cc. cane sugar; +10 cc. invertase working soln. contg. 0.007 g. invertase; + other additions as noted.

**No. 15**: +80 cc. water; $p_{H^+}$ = 6.5, 6.5 (by rosolic acid).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 5.8 | 9.6 | 12.0 | 14.8 | 21.2 | 25.5 |
| $K \times 10^4$ | 36 | 31 | 27 | 25 | 27 | 26 |

**No. 16**: +70 cc. water; +10 cc. 1.0 *M* NaCl; $p_{H^+}$ = 6.4, 6.4 (by rosolic acid).

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 |
|---|---|---|---|---|---|---|
| Mg. cane sugar inverted | 4.5 | 7.6 | 9.9 | 12.6 | 16.8 | 20.4 |
| $K \times 10^4$ | 27 | 24 | 21 | 21 | 20 | 19 |

**No. 17**: +30 cc. water; +50 cc. 1.0 *M* NaCl; $p_{H^+}$ = 6.5, 6.5 (by rosolic acid).

| Minutes | 15 | 30 | 45 | 60 | 90 |
|---|---|---|---|---|---|
| Mg. cane sugar inverted | 2.4 | 4.3 | 6.3 | 8.2 | 10.8 |
| $K \times 10^4$ | 14 | 13 | 13 | 13 | 12 |

## Discussion of Salt Effect.

A consideration of the above facts shows that the effect of the sodium chloride is twofold: first, it causes an increase in the concentration of hydrogen ion when the concentration of hydrochloric acid is greater than 0.0001 molar; secondly, it causes a decrease in the activity of the invertase, provided that in each case the concentration of hydrogen ion is the same. This latter effect varies with the index of the solution and with the amount of sodium chloride added. It is least at the optimum and increases as we depart on either side from this optimum.

This shows that the use of buffers in large concentrations for regulating the index of the solution introduces more or less of an error if it is desired to get the maximum activity of invertase corresponding to a given concentration of hydrogen ion.

The foregoing results also disclose another very interesting feature upon examining the velocity coefficients calculated from them by means of the equation $k = \frac{1}{t} \log \frac{a}{a - x}$. If the successive velocity coefficients for any given test are considered, it will be noticed that one of the three following relationships applies: (1) the coefficients steadily increase in value; (2) they do not change; (3) they steadily decrease. That is to say, the acceleration may be either positive, zero, or negative during the course of the reaction.

TABLE V.

Values of $k \times 10^4$ when $p_{H^+} = 3.1$.

| Minutes | 15 | 30 | 45 | 60 | 90 | 120 | |
|---|---|---|---|---|---|---|---|
| No NaCl | 73 | 73 | 73 | 75 | 79 | 80 | Increase of $k$ |
| 0.1 $M$ NaCl | 64 | 60 | 60 | 62 | 60 | 60 | No change of $k$ |
| 0.5 $M$ NaCl | 36 | 29 | 25 | 24 | 22 | 21 | Decrease of $k$ |
| 2.0 $M$ NaCl | 12 | 12 | 8 | 8 | 6 | 5 | Decrease of $k$ |
| Values of $k \times 10^4$ when $p_{H^+} = 4.5$. | | | | | | | |
| No NaCl | 86 | 87 | 94 | 94 | 99 | 106 | Increase of $k$ |
| 0.1 $M$ NaCl | 87 | 87 | 89 | 94 | 97 | 122 | Increase of $k$ |
| 0.5 $M$ NaCl | 86 | 85 | 91 | 94 | 106 | 122 | Increase of $k$ |
| 2.0 $M$ NaCl | 67 | 74 | 83 | 83 | 90 | 91 | Increase of $k$ |
| Values of $k \times 10^4$ when $p_{H^+} = 6.5$. | | | | | | | |
| No NaCl | 36 | 31 | 27 | 25 | 27 | 26 | Decrease of $k$ |
| 0.1 $M$ NaCl | 27 | 24 | 21 | 21 | 20 | 19 | Decrease of $k$ |
| 0.5 $M$ NaCl | 14 | 13 | 13 | 13 | 12 | 11 | Decrease of $k$ |

The progressive change in the value of $k$ in those cases where it occurs cannot be ascribed to experimental error due to mutarotation, as Hudson[1] has pointed out is the case with Henri's results,[2] since a reduction method was used for the estimation of the amount of sugar inverted. Nor can it be ascribed to a change in the concentration of hydrogen ion of the solution taking place during the hydrolysis of the sugar, because repeated measurements in this regard showed that the concentration of hydrogen ion remained constant throughout the whole course of the inversion.

O'Sullivan and Tompson[3] have noticed an increase in the value of $k$,

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[1] THIS JOURNAL, 30, 1564 (1908).

[2] *Z. physik. Chem.*, 39, 194 (1902).

[3] *Loc. cit.;* Tableaux 24–29.

up to a certain point, about 80% of inversion and after that a decrease. Sörensen gives five examples of increasing values and one of decreasing. Hudson's results[1] show a constant velocity coefficient and likewise those of A. Taylor,[2] while Michaelis and Davidsohn[1] find increasing values.

It does not seem possible at present to find a generalization that will characterize this phenomenon of acceleration so as to bring into harmony the observations of the above investigators, because as is evident from our experiments the acceleration is affected by several variables. Thus in the absence of sodium chloride, a change in the concentration of hydrogen ion causes a change in the acceleration. In the presence of sodium chloride, if the concentration of hydrogen ion is kept constant, varying the concentration of the sodium chloride may or may not affect the acceleration.

For these reasons the results of different authors cannot be compared strictly in this regard, although the preponderance of evidence seems to show that an increase in the value of $k$ with the progress of the reaction is what most investigators have encountered in studying the hydrolysis of cane sugar by means of invertase. These considerations obviously nullify the question put forth by Michaelis and Menton[3] as to the reliability of the constancy of Hudson's coefficients.

## Measurement of the Concentration of Hydrogen Ion.

In measuring the concentration of hydrogen ion, the electromotive force method[4] and the colorimetric method[5] were used.

In this regard it is to be pointed out that each method has certain inherent limitations. It is not possible to employ the electromotive force method when the index of the solution has a value lying between 3.7 and 9.0 and the solution is free from salt. This is on account of the fact that the resistance of the solution is so great under these circumstances that a fairly sensitive galvanometer (*i. e.*, one having a sensitivity of 280 megohms) scarcely gives a noticeable deflection when the circuit is closed. If, however, the solution contains a small amount of salt there is no trouble in making measurements in the region mentioned. Whether the index which is obtained in the presence of salt is exactly the same as the index of the solution without the salt is still a matter to be determined experimentally. It is usually assumed that the formula of Arrhenius[6] holds, and that the addition of a neutral salt with an ion in common causes a repression of the ionization of the acid.

When the concentration of hydrogen ion is determined in the presence

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[1] *Loc. cit.*

[2] *J. Biol. Chem.*, 5, 405 (1909).

[3] *Biochem. Z.*, 49, 333 (1913).

[4] Nernst, *Z. physik. Chem.*, 4, 129 (1889).

[5] Sörenson, *Loc. cit.*

[6] *Z. physik. Chem.*, 31, 204 (1899).

of salts by the colorimetric method the results vary more or less widely from the truth,[1] the amount of variation depending upon the particular indicator used and the kind and amount of salt present. Proteins and chloroform or toluene, used to prevent the growth of microörganisms, also have more or less influence.

The electromotive force measurements were all made at a temperature of 25°. The zero method was employed and the voltages obtained by means of a Leeds & Northrup potentiometer and a D'Arsonval galvanometer which had the following characteristics: a sensitivity of 280 megohms, a period of 2.5 seconds, and a total resistance of 444 ohms. As primary standard of potential a Weston standard cell, which had been calibrated by the Bureau of Standards, was used.

The voltages are those given by the combination $H_2$ — Pt — $H^+$ — 3.5 $M$ KCl — 0.1 $M$ KCl — HgCl — Hg after correcting for the contact potential by Bjerrum's[2] method of extrapolation, which is to add, to the voltage obtained by using 3.5 $M$ KCl as the salt bridge, the difference between this voltage and that obtained by using 1.75 $M$ KCl as the salt bridge.

From the extrapolated voltage, the concentration of hydrogen ion was calculated by the equation

$$\log \text{conc. } H^+ = \frac{0.335 - \pi}{0.591} \text{ (valid only for } 25^\circ\text{)},$$

where $\pi$ is the voltage obtained by extrapolation of the observed voltages according to Bjerrum's method.

The platinum electrodes were always checked up before use, by measuring the voltage given by a solution of 0.1 $M$ HCl. This is a very important precaution, because if, as often happens, the observed voltage under these circumstances differs by more than a millivolt from the calculated, the platinum electrode (or possibly the calomel cell) is not working properly.

**Adjustment of $p_H^+$.**—Attention must be called to the great influence that the character of the water used has in the adjustment of $p_H^+$ in the region $p_H^+ = 4.5$ to 6.0 by means of dilute hydrochloric acid. If buffers are used for this adjustment, there is no difficulty in regulating the concentration of hydrogen ion, but to offset this, there is introduced the salt effect due to the buffer.

While pure water has theoretically an index of 7.0 at 25°, it was not found possible to prepare any water having such an index. The ordinary distilled water used in this laboratory has given a fairly constant index around 6.2. When this water was distilled over Nessler solution and condensed through a tin condenser, the main portion of the distillate had an index of 5.8. When the water was distilled over alkaline permanganate, the index obtained was 5.8. Furthermore, when the water

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[1] Cf. Sörensen, *Compt. rend. Laboratoire de Carlsberg*, Vol. 9 (1910).

[2] *Z. physik. Chem.*, **53**, 428 (1905).

was distilled over alkaline permanganate and then over phosphoric acid, an index of 5.8 was obtained. In these tests the index was determined by using *p*-nitrophenol as indicator and mixtures of citrate solution and 0.1 molar sodium hydroxide as the comparison standards.[1]

The following analyses show that the low index, in the case of the ordinary distilled water, and of the water distilled over Nessler solution, is probably caused by the carbonic acid present, although the observed indices of 6.2 and 5.8 are, respectively, higher than the calculated values of 5.2 and 5.5 figured from the amounts of carbonic acid present.

TABLE VI.

| | (A). Ordinary distilled water. | (B). Water distilled over Nessler solution. |
|---|---|---|
| Total solids | 7.6 mg. per liter | 4.0 mg. per liter |
| Loss upon ignition | 4.8 mg. per liter | 1.2 mg. per liter |
| Fixed solids | 2.8 mg. per liter | 2.8 mg. per liter |
| Total $NH_3$ | 0.58 mg. per liter | 0.03 mg. per liter |
| Free $CO_2$ | 6.0 mg. per liter | 2.2 mg. per liter |
| $p_{H^+}$ (*p*-nitrophenol) | 6.2 | 5.8 |
| Conductivity × $10^6$ recip. ohms | 2.5 | 1.5 |

The results obtained by using the above two samples of water show the influence that the character of the water has upon the adjustment of $p_{H^+}$ at extreme dilutions of hydrochloric acid, as is evidenced by the following table. For indices less than 4.0 this effect disappears.

TABLE VII.

| Cc. HCl in 100 cc. | Molarity of HCl. | Calc. values for $p_{H^+}$. | Obs. values for $p_{H^+}$. (A). | Obs. values for $p_{H^+}$. (B). |
|---|---|---|---|---|
| 0.1 cc. 0.01 *M* | 0.00001 | 5.00 | 5.85 | 5.65 |
| 0.2 | 0.00002 | 4.70 | 5.7 | 5.4 |
| 0.4 | 0.00004 | 4.40 | 5.5 | 4.9 |
| 0.6 | 0.00006 | 4.22 | 5.3 | 4.4 |
| 0.8 | 0.00008 | 4.10 | 5.0 | 4.1 |
| 1.0 | 0.0001 | 4.0 | 4.6 | 4.0 |
| 2.0 | 0.0002 | 3.7 | 4.1 | 3.8 |
| 4.0 | 0.0004 | 3.4 | 3.4 | 3.4 |
| 0.6 cc. 0.1 *M* | 0.0006 | 3.22 | 3.2 | 3.2 |
| 0.8 | 0.0008 | 3.10 | 3.1 | 3.1 |
| 1.0 | 0.001 | 3.00 | 3.0 | 3.0 |
| 2.0 | 0.002 | 2.71 | Observed and calculated values agree from here down | |
| 4.0 | 0.004 | 2.41 | | |
| 6.0 | 0.006 | 2.23 | | |
| 8.0 | 0.008 | 2.11 | | |
| 2.0 cc. 0.5 *M* | 0.01 | 2.01 | | |
| 4.0 | 0.02 | 1.71 | | |
| 6.0 | 0.03 | 1.55 | | |
| 8.0 | 0.04 | 1.41 | | |
| 9.0 | 0.045 | 1.37 | | |
| 1.0 cc. 1.0 *M* | 0.05 | 1.33 | | |

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Cf. Sörensen, *Loc. cit.*

In his admirable work on the kinetics of invertase action, Hudson[1] obtained a value for the optimum of invertase action at $p_{H}^{+} = 3.1$, whereas that obtained by Sörensen,[2] Michaelis and Davidsohn,[2] and the present authors is in the neighborhood of $p_{H}^{+} = 4.2$ to 4.4. This difference of Hudson's is due very likely, in part at least, to the fact that Hudson calculated the concentrations of hydrogen ion from the molarity of the hydrochloric acid present. Such a calculation introduces more or less of an error when the actual index of the solution lies between 4.0 and 9.0, as will be further seen upon examination of the following table, showing the calculated and the observed values in the case of dilute sodium hydroxide solutions.

TABLE VIII.

| Cc. NaOH in 100 cc. | Molarity of NaOH. | Calc. values for $p_{H}+$. | Obs. values for $p_{H}+$. Ordinary distilled water. |
|---|---|---|---|
| 0.1 cc. 0.01 *M* | 0.00001 | 9.14 | |
| 0.2 | 0.00002 | 9.44 | |
| 0.3 | 0.00003 | 9.62 | 6.3 (*p*-nitrophenol) |
| 0.4 | 0.00004 | 9.74 | |
| 0.5 | 0.00005 | 9.84 | |
| 0.6 | 0.00006 | 9.92 | 7.1 (rosolic acid) |
| 0.7 | 0.00007 | 9.99 | |
| 0.8 | 0.00008 | 10.04 | 7.8 (rosolic acid) |
| 0.9 | 0.00009 | 10.09 | |
| 1.0 | 0.0001 | 10.14 | 8.7 (phenolphthalein) |

The above differences between the calculated and the observed values for $p_{H}^{+}$ firmly establish the necessity of actually measuring the concentration of hydrogen ion instead of just calculating it from the molarity of the acid or alkali used.

## Measurement of Amount of Cane Sugar Inverted.

Owing to the fact that a concentration of cane sugar of 5 g. per liter was used in this work, it was not practicable to use the polariscope to measure the amount of inversion, nor to use any of the numerous sugar tables. Therefore, a modified Defren's method[3] was used as follows:

**Reagents.**—34.64 g. of copper sulfate dissolved in water, 0.5 cc. conc. sulfuric acid added, and made up to 500 cc. 178 g. sodium potassium tartrate and 50 g. of sodium hydroxide dissolved in water and diluted to 500 cc.

**Determination.**—Mix 15 cc. of each of the above reagents in an Erlenmeyer flask having a capacity of 250 to 300 cc., dilute with 65 cc. of distilled water, then add 10 cc. of the sample undergoing hydrolysis, place in boiling water bath and allow to stand for exactly fifteen minutes. Remove the flask from the bath and filter at once (using moderate suction) through asbestos specially prepared as usual for this purpose; wash the cuprous

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[1] THIS JOURNAL, **32**, 1220 (1910).

[2] *Loc. cit.*

[3] Cf. Defren, THIS JOURNAL, **18**, 749 (1896).

oxide with boiling distilled water until free from alkali. Dissolve the cuprous oxide in 6 *M* nitric acid, etc., and determine the copper by means of potassium iodide and sodium thiosulfate. It is necessary to run a blank control to determine the correction to be applied for the effect of the Defren method on cane sugar itself. Under the conditions of our experiments we found the reducing effect of 50 mg. cane sugar to be equivalent to 2.8 mg. cupric oxide, or 1 mg. cane sugar inverted.

From the weight of cupric oxide corresponding to the weight of copper found, the amount of cane sugar inverted was calculated according to the following sugar table constructed by the authors as hereinafter described:

TABLE IX.

| Mg. CuO. | Mg. cane sugar inverted. | Mg. CuO. | Mg. cane sugar inverted. |
|---|---|---|---|
| 10 | 3.6 | 70 | 27.2 |
| 20 | 7.2 | 80 | 31.5 |
| 30 | 11.0 | 90 | 35.9 |
| 40 | 14.9 | 100 | 40.4 |
| 50 | 18.9 | 110 | 45.0 |
| 60 | 23.0 | 120 | 49.7 |

## Construction of Sugar Table.

280 cc. of sugar solution containing 59.998 g. cane sugar were made up to 300 cc. with "stock solution" of invertase and 0.2 cc. 0.1 *M* hydrochloric acid, which brought the index of the solution down to 4.8. The flask containing this solution was placed in the thermostat, which was maintained at the usual temperature of 37° ± 0.01° C. A 50 cc. sample was withdrawn at 15, 30, 60, 120 and 180 minutes, respectively, and run into 16.7 cc. of 0.2 *M* sodium carbonate solution, as recommended by Hudson for arresting inversion and eliminating the effect due to mutarotation.

10 cc. or 20 cc. were withdrawn from this 66.7 cc. and diluted twenty or thirty times, and a Defren's determination, as already described, run on 10 cc. portions from the diluted solution. The rest of the 66.7 cc. was used to determine the amount of inversion by means of the polariscope in the first series of determinations, and by means of the saccharimeter in the second series.

TABLE X.

Conversion of Polariscope Readings to Percentage Inversion.

| Time. Mins. | Readings at 30°. | | | Average. | Angle of inversion. | % Inversion. |
|---|---|---|---|---|---|---|
| Start | 21.90 | (calc.) | ... | ... | ... | ... |
| 15 | 20.36 | 20.40 | ... | 20.38 | 1.52 | 5.52 |
| 30 | 18.83 | 18.86 | 18.86 | 18.85 | 3.05 | 11.08 |
| 60 | 16.13 | 16.15 | 16.14 | 16.14 | 5.76 | 20.96 |
| 120 | 11.15 | 11.18 | 11.21 | 11.18 | 10.72 | 38.94 |
| 180 | 7.38 | 7.36 | 7.36 | 7.37 | 14.53 | 52.78 |
| | —5.63 | (calc.) | | | | |

TABLE X (*continued*).

Conversion of Saccharimeter Readings (Ventzke) to Percentage Inversion.

| Time. Mins. | Readings at 35°. | | Average. | Angle of inversion. | % Inversion. |
|---|---|---|---|---|---|
| Start | 62.8 | 62.8 | 62.8 | .. | ... |
| 15 | 58.6 | 58.6 | 58.6 | 4.2 | 5.41 |
| 30 | 55.0 | 55.0 | 55.0 | 7.8 | 10.04 |
| 60 | 48.2 | 48.2 | 48.2 | 14.6 | 18.80 |
| 120 | 35.2 | 35.2 | 35.2 | 27.6 | 35.53 |
| 180 | 24.2 | 24.2 | 24.2 | 38.6 | 49.69 |
| 240 | 14.5 | 14.5 | 14.5 | 48.3 | 62.18 |
| 2220 | —14.3 | —14.3 | —14.3 | 77.1 | 99.26 |
| | —14.6 | (calc.) | | | |

TABLE XI.

Determination of Amount of Cane Sugar Inverted Corresponding to Cupric Oxide.

| Dilution of sample from 66.7 cc.[1] | Equiv. grams cane sugar in 10 cc. used for Defren's. | Percentage inversion. | Grams CuO. | Dilution of sample from 66.7 cc.[1] | Equiv. grams cane sugar in 10 cc. used for Defren's. | Percentage inversion. | Grams CuO. |
|---|---|---|---|---|---|---|---|
| 10 cc. made up to 100 | 0.1499 | 5.52 | 0.0227 | 20 cc. made up to 600 | 0.0500 | 5.41 | 0.0085 |
| 200 | 0.0750 | 11.08 | 0.0230 | | 0.0500 | 10.04 | 0.0155 |
| 250 | 0.0580 | 20.92 | 0.0328 | | 0.0500 | 18.80 | 0.0254 |
| 200 | 0.0750 | 38.94 | 0.0748 | | 0.0500 | 35.53 | 0.0456 |
| 200 | 0.0750 | 52.78 | 0.0982 | | 0.0500 | 49.69 | 0.0633 |
| | | | | | 0.0500 | 62.18 | 0.0795 |
| | | | | | 0.0500 | 99.26 | 0.1200 |

If these results are expressed in the form of an equation, derived by the method of least squares, we get

$$\text{Cane sugar inverted} = 0.351\ W + 0.000527\ W^2$$

where W is the weight of cupric oxide obtained, the values of W varying from 8 to 120 mg.

The values in the sugar table already given were calculated by the use of this equation.

The agreement between the values obtained by experiment and those calculated by the use of the above equation is shown in Table XII.

TABLE XII.

| | Mg. cane sugar inverted. | | | | Mg. cane sugar inverted. | | |
|---|---|---|---|---|---|---|---|
| Mg. CuO. | Obs. | Calc. | Diff. | Mg. CuO. | Obs. | Calc. | Diff. |
| 8.5 | 2.7 | 3.0 | +0.3 | 45.6 | 17.8 | 17.1 | —0.7 |
| 15.5 | 5.0 | 5.7 | +0.7 | 63.3 | 24.8 | 24.3 | —0.5 |
| 22.7 | 8.3 | 8.3 | ... | 74.8 | 29.2 | 29.2 | ... |
| 23.0 | 8.3 | 8.4 | +0.1 | 79.5 | 31.1 | 31.2 | +0.1 |
| 25.4 | 9.4 | 9.2 | —0.2 | 98.2 | 39.6 | 39.5 | —0.1 |
| 32.8 | 12.1 | 12.1 | ... | 120.0 | 49.6 | 49.6 | ... |

## Summary.

The concentration of hydrogen ion remains constant throughout the whole course of the inversion of cane sugar by means of invertase.

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[1] Of these diluted solutions, 10 cc. were used for Defren's.

At the optimum of invertase action, the salt effect of the sodium chloride seems to approach zero, and as we depart either side from the optimum, we get an increasing salt effect.

The use of buffers (tampons) for regulating the concentration of hydrogen ion introduces a certain salt effect. The most satisfactory region for using buffers in invertase velocity measurements is in the neighborhood of the optimum zone where the salt effect is a minimum.

In the region of enzyme activity it is necessary to measure the concentration of hydrogen ion, and it is not permissible to calculate it from the molarity of acid used.

The addition of sodium chloride to solutions of hydrochloric acid causes an increase in the concentration of hydrogen ion as measured by the electromotive force method and by the hydrolysis of cane sugar solutions.

NEW YORK, N. Y.

## CORRECTION.

**The Dissociation of Carbon Oxysulfide.**—Through the kindness of Professor J. Sakurai, of the University of Tokio, my attention has been called to an important error in sign in the recent paper by Lewis and Lacey.[1] There it is stated, "It is evident that with diminishing temperature the equilibrium is shifted in favor of CO." This should have read "with increasing temperature." Consequently, the van't Hoff equation gives for the heat of formation of COS from liquid sulfur and carbon monoxide not — 11000 but + 11000 calories. For this reaction Berthelot found — 4000 and Thomsen + 10000 cal. Our value therefore corroborates that of Thomsen, and we may now feel safe in using this value in the calculation of the free energy of the reaction at standard temperature. This calculation will be made in another place. GILBERT N. LEWIS.

## NEW BOOKS.

**Laboratory Manual to Accompany "A Course in General Chemistry."** By WM. MCPHERSON AND WM. E. HENDERSON. Ohio State University. Pp. v + 140. Ginn and Co., 1915. Price, 60 cents.

The role this manual plays is well stated in the preface by the authors: "For one who sets about the task of arranging an experimental course for the beginner, there remains little opportunity for originality or invention. His problem is rather one of selection. Accordingly, this laboratory manual lays no claim to originality, either in method or in content." When used in connection with the text-book, by the same authors, it proves to be a fairly satisfactory guide for an elementary course. The introductory experiments are not well chosen. For example, the student infers from the experiment that when salt is dissolved in water the change

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[1] THIS JOURNAL, 37, 1981 (1915).