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KINETICS OF INVERTASE ACTION.

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The kinetics of the hydrolysis of cane sugar in the presence of invertase has received considerable attention from various investigators. O'Sullivan and Tompson[1] concluded that the reaction was of the first order like the hydrolysis in the presence of acids. On the other hand, Duclaux[2] disagreed with O'Sullivan and Tompson on the ground that the rate of inversion, in the case of acids as catalyst, was proportional to the concentration of the sugar, while in the case of invertase this did not seem to be so. Brown[3] found that the velocity coefficients, calculated on the unimolecular basis, increased as the reaction proceeded. Barendrecht[4] suggested a hypothesis that enzymes emit a certain kind of radiation which is absorbed by the substrate and its hydrolytic products and thereby giving rise to the enzyme effect. In the case of the inversion of cane sugar, the invert sugar also absorbs part of the radiation and thereby gradually diminishes the amount of radiation which otherwise would be absorbed by the cane sugar. Due to this effect of the invert sugar, the hydrolysis of the cane sugar does not follow strictly the simple mass law. Henri[5] also found that the velocity coefficients for the inversion of sugar by invertase were not sufficiently constant to warrant the claims that the reaction is one of the first order. He[6] suggested that due to the colloidal nature of enzymes, the reaction belongs to a two-phased system and therefore the simple mass law is not strictly applicable.

Hudson[7] criticized Henri in that the latter had not made allowance for the mutarotation of the invert sugar in measuring the extent of the hydrolysis by means of the polariscope. When this effect was taken into account Hudson found, in the cases he investigated, that the hydrolysis of cane sugar in the presence of invertase gave velocity coefficients that were constant when calculated by the unimolecular formula. He therefore claims to have confirmed the conclusions of O'Sullivan and Tompson.

Michaelis and Menton[8] grant that Hudson is justified in his criticism of Henri's neglect to take into account the effect of mutarotation, but

[1] *J. Chem. Soc.*, **57**, 834 (1890).
[2] *Ann. Inst. Pasteur*, **12**, 96 (1898).
[3] *J. Chem. Soc.*, **81**, 375 (1902).
[4] *Z. physik. Chem.*, **49**, 456 (1904); *Biochem. J.*, **7**, 553 (1913).
[5] *Ibid.*, **39**, 215 (1902).
[6] *Z. physik. Chem.*, **51**, 19 (1905).
[7] THIS JOURNAL, **30**, 1160, 1564 (1908).
[8] *Biochem. Z.*, **49**, 333 (1913).

question whether it is of such far-reaching importance as claimed by him. They disagree with Hudson in attempting to express the reaction of inversion by invertase as a simple logarithmic function of the sugar concentration and point out that the idea of Henri[1] that the substrate and enzyme form an intermediate compound is sound. Michaelis and Menton have elaborated this idea and claim that the velocity of inversion is proportional to the concentration of the intermediate compound.

Nelson and Griffin[2] have shown that the inversion of cane sugar through invertase action is a two-phased reaction, thereby confirming the hypothesis of Henri in this respect. The fact that the reaction is one of a two-phased system places it in the class of heterogeneous catalysis and therefore different from the inversion of sugar by acids which belongs to the class of homogeneous catalysis. This difference between these two reactions has an important significance in connection with the misunderstanding which has crept into the literature, based chiefly upon the statement of Hudson in his apparent confirmation of O'Sullivan and Tompson's conclusion that the two reactions are alike. Thus quoting Arrhenius,"[3]

"This observation is very important as it shows again that the supposed difference in action of organic products (enzymes) and inorganic substances (acids) is not a real one."

Czapek[4] also makes the statement:

"In this way it was clearly shown that the inversion of cane sugar by invertase is just as much a monomolecular reaction as the parallel reaction of cane sugar inversion by means of acids."

Bayliss[5] was probably one of the first really to consider the formation of the intermediate compound from the enzyme and substrate, preliminary to the chemical change brought about by the enzyme, as adsorption, and that the rate of enzyme action is a function of the amount of adsorption compound in existence at any particular time.

Herzog[6] has based a theory for the kinetics of enzyme action upon the diffusion velocity of the substrate. He has shown that if it is assumed that the velocity of hydrolysis is proportional to the square root of the rate of diffusion, as calculated from the reciprocal of the viscosity of the solution, then the velocity of inversion in the case of the experiments of Henri can be calculated. Henri,[7] however, has shown that this view is not in accord with all the facts.

Lewis[8] considers enzyme action as being dependent on the amount of

[1] *Compt. rend.*, **135**, 916 (1902).
[2] THIS JOURNAL, **38**, 1109 (1916).
[3] "Theories of Solutions," 1912 Ed., p. 114.
[4] "Chemical Phenomena of Life," Harpers, 1911.
[5] *Proc. Roy. Soc. London*, (*B*) **84**, 90 (1911).
[6] *Z. physiol. Chem.*, **41**, 420 (1904).
[7] *Z. physik. Chem.*, **51**, 27 (1905).
[8] "A System of Physical Chemistry," Ramsay Series, 1916, Vol. I, p. 509.

substrate adsorbed by the enzyme, and, on account of the Brownian movement, independent of the rate of diffusion of substrate. Nernst,[1] however, has pointed out that in a heterogeneous reaction the rate of diffusion must play an important part in determining the velocity of the reaction. The results of Nelson and Griffin, that invertase adsorbed by charcoal or aluminium hydroxide has the same catalytic effect as the same amount of invertase in solution, make it evident that the Brownian movement can have no effect, since it would not be expected that invertase adsorbed to the charcoal at the bottom of the reaction vessel would be still in rapid motion. Consequently, it must be assumed that diffusion of the sugar to the invertase is one of the controlling factors. Noyes and Whitney[2] have shown that the solution of a solid, the velocity of which is dependent on diffusion, follows a law which is of the same form as the unimolecular law. It can be understood then why the inversion of cane sugar by invertase could be dependent upon the rate of diffusion and still appear to follow the unimolecular law.

It is evident from the above review of the opinions of previous investigators, who have studied the kinetics of invertase action, that its true nature is still an unsettled question, and that more experimental data are desirable. The following work was undertaken in hopes that some of these necessary data might be obtained. Although the data obtained do not answer the question completely, they do indicate, however, the limitations and incorrectness of some of the theories proposed by some of the previous investigators.

It has also been found, at least in the experiments of this investigation, that:

1st. The velocity of inversion is directly proportional to the concentration of the invertase.

2nd. The velocity is nearly independent of the concentration of the cane sugar in the more concentrated sugar solutions, while in very dilute sugar solutions the velocity increases with the increase of concentration of the substrate, and finally reaches a maximum.

3rd. The results obtained agree with the heterogeneous reaction view and contradict the claim that the kinetics of invertase action conform to the unimolecular law for homogeneous reactions.

4th. Adsorption is one of the controlling factors in the kinetics of invertase action, and the velocity of inversion curve, where the concentration of cane sugar is used as abscissas, has the same general shape as adsorption curves as suggested by Henri.[3]

5th. The inversion of cane sugar by invertase is a different type of

[1] *Z. physik. Chem.*, **47**, 52 (1904).

[2] *Ibid.*, **23**, 689 (1897).

[3] *Ibid.*, **51**, 27 (1905).

heterogeneous catalytic reaction from the dissociation of stibine into antimony and hydrogen in the presence of metallic antimony, and the dissociation of molecular hydrogen into atomic hydrogen in the presence of heated metals.

Experimental.

The first 15 experiments were carried out in such a way as to permit the concentration of cane sugar and invertase to be varied while the hydrogen-ion concentration was maintained at the optimum, about $c_{H+} = 10^{-4.5}$. Experiments 16–21 differed from the first 15 in that a different preparation of invertase, B, was used and the hydrogen-ion concentration was somewhat lower, $c_{H+} = 10^{-5.5}$. Experiments 22 and 23 differed from Experiments 16–21 in having the optimum hydrogen-ion concentration as in the first 15. Experiment 24 was similar to Experiments 16–21; invertase A was used instead of B. The results from Experiments 1–24 were calculated as per cent. inverted in a given time, by dividing the difference between the initial angle at the time t by the total angle, the latter being the sum of the initial angle and the angle at 100% inversion. The velocity coefficients, k, were calculated by applying the unimolecular equation, $k = 1/t \log 1/1 - x$, where x is the fraction, %/100, inverted at the time t. The Sorensen symbol, $p_H{}^+$, for the negative exponent of 10 was used in indicating the hydrogen-ion concentration.

TABLE I.
Composition of Solutions.

Experiment No.	1	2	3	4	5	6
Cc. invertase per 100 cc. soln.	6	5	4	3	2	6
G. cane sugar per 100 cc. soln.	5	5	5	5	5	10
Acidity, $p_H{}^+$	4.5	4.5	4.5	4.45	4.45	4.58

7	8	9	10	11	12	13	14
5	4	3	2	6	5	4	3
10	10	10	10	20	20	20	20
4.5	4.58	4.5	4.58	4.42	4.45	4.52	4.5
15	16	17	18	19	20	21	22
2	2	1	2	1	2	1	1
20	5	5	10	10	20	20	10
4.45	5.47	5.43	5.5	5.35	5.44	5.45	4.65
23	24	25	26	27*a*	27*b*	28*a*	28*b*
2	3	4	4	4	4	4	4
10	10	20	10	5	5	4	4
4.6	5.46	4.3	4.3	4.37	4.38	4.26	4.34
29*a*	29*b*	29*c*	30*a*	30*b*	31*a*	31*b*	
4	4	4	4	4	4	4	
2	2	2	1	1	0.4	0.4	
4.32	4.33	4.34	4.34	4.39	4.44	4.38	

Invertase A was used in Experiments 1–15 and 24; Invertase B in Experiments 16–23; and Invertase A diluted in Experiments 25–31.

VELOCITY OF INVERSION.

Time in mins. t.	Observed rotation.	Per cent. inversion.	k.	Time in mins. t.	Observed rotation.	Per cent. inversion.	k.
	Experiment No. 1.				Experiment No. 2.		
0	6.06°	.....	.....	0	6.07°	.....	.....
8	5.28°	10.08	0.00577	10	5.25°	10.57	0.00485
18	4.35°	22.09	0.00602	20	4.48°	20.49	0.00498
35	2.90°	40.83	0.00651	41	2.99°	39.69	0.00536
55	1.50°	58.91	0.00702	68	1.44°	59.67	0.00580
90	—0.12°	79.85	0.00773	110	—0.16°	80.28	0.00641
160	—1.31°	95.22	0.00825	185	—1.30°	94.98	0.00702
∞	—1.68°	.....	.....	∞	—1.69°	.....	.....
	Experiment No. 3.				Experiment No. 4.		
0	6.06°	.....	.....	0	6.06°	.....	.....
12	5.28°	10.05	0.00383	16	5.23°	10.67	0.00306
24	4.50°	10.20	0.00406	32	4.48°	20.31	0.00308
51	2.97°	39.82	0.00432	60	3.28°	35.73	0.00320
85	1.41°	59.93	0.00467	90	2.09°	51.03	0.00345
130	—0.02°	78.35	0.00511	115	1.31°	61.05	0.00356
234	—1.32°	95.10	0.00560	180	—0.18°	80.21	0.00391
∞	—1.70°	.....	.....	318	—1.38°	95.63	0.00428
				∞	—1.72°	.....	.....
	Experiment No. 5.				Experiment No. 6.		
0	6.06°	.....	.....	0	12.02°	.....	.....
24	5.27°	10.14	0.00193	14	10.73°	8.32	0.00270
49	4.49°	20.15	0.00199	30	9.24°	17.92	0.00286
100	3.04°	38.77	0.00213	70	5.84°	39.85	0.00315
165	1.52°	58.28	0.00230	120	2.42°	61.90	0.00349
265	—0.03°	78.18	0.00249	185	—0.48°	80.59	0.00385
450.5	—1.28°	94.22	0.00275	320	—2.71°	94.97	0.00406
∞	—1.73°	.....	.....	∞	—3.49°	.....	.....
	Experiment No. 7.				Experiment No. 8.		
0	12.05°	.....	.....	0	12.04°	.....	.....
22	10.33°	11.08	0.00232	20	10.75°	8.31	0.00188
40	8.92°	20.15	0.00244	45	9.14°	18.69	0.00200
90	5.34°	43.21	0.00273	105	5.65°	41.17	0.00219
138	2.58°	60.98	0.00296	175	2.40°	62.11	0.00241
215	—0.42°	80.30	0.00328	265	—0.39°	80.09	0.00265
373	—2.71°	95.04	0.00350	450	—2.68°	94.85	0.00286
∞	—3.48°	.....	.....	∞	—3.48°	.....	.....
	Experiment No. 9.				Experiment No. 10.		
0	11.99°	.....	.....	0	11.99°	.....	.....
33	10.51°	9.57	0.00132	50	10.44°	10.01	0.00092
70	8.82°	20.49	0.00142	100	8.87°	20.14	0.00098
150	5.57°	41.50	0.00155	221	5.47°	42.09	0.00107
250	2.33°	62.44	0.00170	315	3.30°	56.10	0.00114
376	—0.40°	80.09	0.00186	345	2.69°	60.04	0.00116
660	—2.75°	95.28	0.00201	570	—0.65°	81.60	0.00129
∞	—3.48°	.....	.....	1365	—3.20°	98.06	0.00125
				∞	—3.50°	.....	.....

VELOCITY OF INVERSION (*continued*).

Time in mins. *t.*	Observed rotation.	Per cent. inversion.	*k.*
	Experiment No. 11.		
0	24.21°	.....	.....
35	21.25°	9.42	0.00123
80	17.61°	21.01	0.00128
159.5	11.75°	39.66	0.00138
265	5.35°	60.02	0.00150
425	—0.94°	80.04	0.00165
785	—5.60°	94.88	0.00164
∞	—7.21°	.....	.....
	Experiment No. 12.		
0	24.19°	.....	.....
45	21.02°	10.09	0.00103
90.5	17.95°	19.87	0.00106
190	11.77°	39.54	0.00115
315	5.46°	59.63	0.00125
514	—0.99°	80.17	0.00137
814	—4.91°	92.64	0.00139
∞	—7.22°	.....	.....
	Experiment No. 13.		
0	24.12°	.....	.....
55	21.07°	9.72	0.000807
125	17.27°	21.84	0.000858
250	11.10°	41.51	0.000932.
392	5.36°	59.80	0.001010
636	—0.99°	80.05	0.001101
1350	—6.02°	96.08	0.001042
∞	—7.25°	.....	.....
	Experiment No. 14.		
0	24.14°	.....	.....
75.5	20.98°	10.07	0.000611
150	17.97°	19.66	0.000634
328	11.44°	40.46	0.000687
530	5.42°	59.64	0.000744
853	—0.87°	79.87	0.000816
1398	—5.04°	92.96	0.000824
1598	—5.63°	94.84	0.000806
∞	—7.25°	.....	.....
	Experiment No. 15.		
0	24.12°	.....	.....
110	21.18°	9.38	0.000389
250	17.51°	21.08	0.000411
490	11.83°	39.19	0.000440
840	5.18°	60.39	0.000479
1418	—1.56°	81.89	0.000523
2812	—6.88°	98.85	0.000690
∞	—7.24°	.....	.....
	Experiment No. 16.		
0	6.13°	.....	.....
10	5.30°	10.56	0.00485
21	4.55°	20.10	0.00464
48	2.99°	39.95	0.00461
91	1.24°	62.21	0.00464
155	—0.11°	79.39	0.00443
330	—1.29°	94.40	0.00379
∞	—1.73°	.....	.....
	Experiment No. 17.		
0	6.02°	.....	.....
20	5.25°	9.91	0.00227
42	4.44°	20.33	0.00235
94	2.91°	40.03	0.00238
170	1.13°	62.93	0.00254
315	—0.81°	87.90	0.00291
450	—1.44°	96.01	0.00311
∞	—1.75°	.....	.....
	Experiment No. 18.		
0	12.07°	.....	.....
16	10.62°	9.28	0.00264
35	8.97°	19.85	0.00275
77	5.94°	39.24	0.00281
140	2.63°	60.44	0.00288
235	—0.21°	78.62	0.00285
435	—2.42°	92.77	0.00262
∞	—3.55°	.....	.....
	Experiment No. 19.		
0	12.06°	.....	.....
35	10.49°	10.06	0.00132
73	8.99°	19.68	0.00130
175	5.43°	42.50	0.00137
285	2.66°	60.26	0.00141
510	—0.59°	81.09	0.00142
∞	—3.54°	.....	.....
	Experiment No. 20.		
0	24.08°	.....	.....
40	20.79°	10.49	0.00120
82	17.49°	21.01	0.00125
190	10.64°	42.84	0.00128
305	5.64°	58.78	0.00126
570	—0.80°	79.31	0.00120
1397	—6.09°	96.18	0.00101
∞	—7.29°	.....	.....

VELOCITY OF INVERSION (*continued*).

Time in mins. *t.*	Observed rotation.	Per cent. inversion.	*k.*
	Experiment No. 21.		
0	24.00°	.....	.....
80	20.74°	10.42	0.000597
160	17.87°	19.59	0.000592
315	13.07°	34.93	0.000592
375	11.50°	39.95	0.000591
675	5.25°	59.92	0.000588
1185	—1.29°	80.83	0.000608
∞	—7.29°	.....	.....
	Experiment No. 22.		
0	12.03°	.....	.....
30	10.33°	10.91	0.00167
60	8.68°	21.50	0.00175
122	5.61°	41.21	0.00189
200	2.45°	61.49	0.00207
305	—0.38°	79.65	0.00227
600	—2.94°	96.08	0.00234
∞	—3.55°	.....	.....
	Experiment No. 23.		
0	12.02°	.....	.....
14	10.41°	10.34	0.00339
28	8.83°	20.49	0.00356
60	5.51°	41.81	0.00392
100	2.27°	62.62	0.00427
156	—0.70°	81.70	0.00473
280	—2.88°	95.70	0.00488
∞	—3.55°	.....	.....
	Experiment No. 24.		
0	12.04°	.....	.....
40	10.58°	9.41	0.00107
85	9.11°	18.88	0.00107
190	6.17°	37.82	0.00109
290	3.83°	52.90	0.00113
350	2.70°	60.17	0.00114
600	—0.43°	80.33	0.00118
1372	—2.85°	95.92	0.00101
∞	—3.48°	.....	.....

Time in mins. *t.*	Observed rotation.	Per cent. inverted.	Mg. CuO. A.	Mg. CuO. B.
	Experiment No. 25.			
0	24.22°	.....	.....	.....
240	23.55°	8.61	49.5	49.7
420	19.53°	14.90	79.7	79.3
540	18.22°	19.07	100.4	99.8
660	17.02°	22.88	116.7	116.7
780	15.86°	26.57	133.4	132.9
∞	—7.25°	.....	.....	.....
	Experiment No. 26.			
0	12.12°	.....	.....	.....
91	10.93°	7.61	45.3	45.5
180	9.77°	15.03	80.4	79.2
240	9.05°	19.63	102.3	101.3
300	8.31°	24.36	123.0	122.8
360	7.67°	28.45	142.7	142.7
∞	—3.52°	.....	.....	.....

Experiment No. 27.

Time.	A. Observed rotation.	A. Per cent. inverted.	B. Observed rotation.	B. Per cent. inverted.
0	6.02°	.....	6.04°	.....
90	4.83°	15.34	4.84°	15.46
135	4.25°	22.81	4.29°	22.55
150	4.09°	24.87	4.12°	24.74.
165	3.94°	26.80	3.94°	27.06
∞	—1.74°	.....	—1.72°	.....

Experiment No. 28.

Time.	Mg. CuO. A.	Per cent. inverted.	Mg. CuO. B.	Per cent. inverted.
50	61.1	11.0	*51.3	8.8
90	96.5	18.5	95.2	18.3
120	121.8	24.1	121.4	24.0
130	128.4	25.5	129.1	25.7
140	136.7	27.2	137.2	27.6

Experiment No. 29.

A.		†B.		C.	
40	77.4	14.4	80.7	15.1	40	81.8	15.4
60	108.9	21.2	114.0	22.2	60	117.5	23.1
80	138.4	27.8	143.5	29.2	75	141.0	28.5
90	150.6	(30.4)	156.5	(31.6)	85	155.9	(31.5)
100	163.3	(33.0)	168.3	(34.0)			

* Time 45 min. instead of 50.

† Time for A and B same.

Experiment No. 30.

Time.	A. Observed rotation.	A. Per cent. inverted.	B. Observed rotation.	B. Per cent. inverted.
20	59.6	10.7	61.9	11.2
40	108.6	21.0	108.9	21.1
50	129.2	25.8	131.2	26.2
60	149.3	(30.1)	151.5	(30.5)
70	167.7	(33.8)	.....	....

Experiment No. 31.

Time.	Mg.CuO. A.	Per cent. inverted.	Mg.CuO. B.	Per cent. inverted.
15	62.2	11.2	60.9	10.9
25	103.0	19.8	100.8	19.4
35	129.4	25.8	129.8	25.8
40	142.9	29.0	144.6	29.4
45	159.2	(32.1)	157.6	(31.8)[1]

[1] The values in parenthesis were obtained by extrapolation.

Discussion of Results.

Varying Velocity Coefficients.—An examination of the values for the velocity coefficients k of Experiments 1–15, 17, and 22–24, shows that in each case the values increase as the inversion proceeds. This agrees with the results obtained by Brown, Michaelis and Menton, and Sorensen, who all found increasing velocity coefficients in the inversion of cane sugar by invertase. It was mentioned in the introductory part, however, that Hudson, Taylor[1] and O'Sullivan and Tompson obtained constant velocity coefficients and that all these investigators considered the reaction one of the first order. The above results are, therefore, contradictory to their conclusions. A more careful examination of the results of O'Sullivan and Tompson makes it apparent that, they too, actually obtained slight increases in the values of the coefficients up to about 80% inversion, and after that a decrease.

It is to be noted that in some of the Experiments, 18, 20 and 21, the values of the velocity coefficients are practically constant and therefore agree with the claims of Hudson. Since Hudson only describes two series of experiments, from the results of which he seems to conclude that k is constant, when calculated upon the unimolecular basis, it seems probable that in the light of the results in Table I, these ks were constant only through coincidence, and were special cases like Experiments 18, 20 and 21.

Fales and Nelson[2] also measured the velocity coefficients for invertase action in dilute sugar solutions, 0.5 g. of cane sugar per 100 cc., and found that if these coefficients were calculated on the unimolecular law basis, they were not always constant in value. This is then another instance where very dilute sugar solutions were used, which might be offered against the claims of Hudson.

Furthermore, the error in Hudson's conclusions can be pointed out in another way besides that of varying velocity coefficients. In Table II the experimental data from Experiments 25–31 has been arranged so as to show the relationship between the velocity of inversion, up to 25% inversion, and the concentration of cane sugar, varying between the limits,

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

[2] THIS JOURNAL, **37**, 2779 (1915).

0.4 and 20 g. per 100 cc. In Fig. 1 this relationship is expressed graphically by using the times for 25% inversion as ordinates and the concentrations of cane sugar as abscissas.

TABLE II.

Experiment No....	25	26	27	28	29	30	31
$p_H{}^+$.....	4.30	4.30	4.38	4.30	4.33	4.36	4.42
G. sugar per 100 cc.	20	10	5	4	2	1	0.4
Time (minutes) for 25% inversion..	728	309	152	126	68	48	34
Conc./time.....	0.0275	0.0324	0.0329	0.0318	0.0294	0.0208	0.0085

The shape of the curve in Fig. 1 shows that when the concentration is low, its slope or tangent increase with the increasing sugar concentration, and only between the concentrations of 5 and 10 g. per 100 cc. does the curve approach a straight line. If the velocity of inversion (below 0.1 molar) for dilute solutions was proportional to the concentration of the cane sugar and the reaction was one of the first order, as claimed by Hudson, then the curve in this region should be a straight line parallel to the x axis.

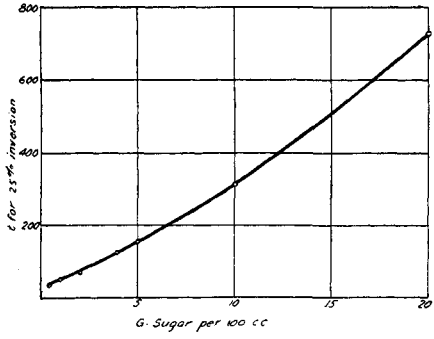

Fig. 1.

Decreasing Velocity Coefficients.—The velocity coefficients in Experiments 18 and 20 show constant or almost decreasing values as the inversion proceeds. Several other investigators have also noticed this fact. One of them, Barendrecht, considers that inversions, where the velocity coefficients are constant or decreasing, are special cases where the hydrogen ion destroys the invertase as the reaction proceeds, and thereby either exactly compensating or else overbalancing the tendency for the constants to increase. This, however, cannot be the case in the above Experiments, 16–21, since they have a much smaller hydrogen-ion concentration than the Experiments 1–15, whose coefficients increase, and if Barendrecht's contentions were valid it would be expected that the more nearly constant or decreasing coefficients would be obtained in the case of the more acid solutions.

Barendrecht also claims that it is dangerous to study the kinetics of enzyme action in solutions as acid as the optimum hydrogen-ion concentration or beyond on account of the destructive action of the hydrogen ion upon the enzyme. He bases his statement on the results of Sorensen,[1] which were carried out at the relatively high temperature of 52°. The

[1] *Comp. Rend. Lab. Carlsberg*, **8**, 1909.

above experiments, however, show that at 37° at least, it is safe to work at the optimum. The results of Fales and Nelson point to similar conclusions. It is evident, therefore, that in the light of the above results, Barendrecht's conjecture as to the reason why constant velocity coefficients may be obtained in the inversion of cane sugar by invertase must be rejected.

Invertase Action as Heterogeneous Catalysis.—It has been shown in the introduction how the view has grown that enzyme action belongs to the class of heterogeneous rather than homogeneous catalysis. The heterogeneous view of enzyme action is supported by the evidence brought forth by Henri, Bayliss, and Nelson and Griffin, together with the results obtained in the present investigation.

Bayliss was one of the first to study enzyme action as an adsorption phenomenon by means of the relation between the velocity and the concentration of the enzyme. He states:[1]

"If the velocity of enzyme action is conditioned in any given case by the amount of adsorption which has taken place, it follows that when the relative concentration of enzyme and substrate is varied, the corresponding change in the rate of action will be an exponential function of the concentration."

The usual simple adsorption equation developed by Freundlich and others has the form

$$x/m = kc^{1/n},$$

in which x is the amount of the substance adsorbed, m is the amount of adsorbent, c the concentration of the substance being adsorbed still in solution, and k and n are constants, the latter usually having a value between 1 and 2, very often the latter value. Since x/m is the amount of substance adsorbed per unit area of surface of the adsorbent, it corresponds to the concentration of the substance in the adsorbed phase. In this way it is possible to write the equation in a somewhat simpler form.

$$x = ky^{1/n},$$

in which x is the concentration of the substance being adsorbed in the adsorbed phase and y the concentration of the same substance in the unadsorbed phase, as for example the solution.

Bayliss considered that this last equation was applicable to expressing the relationship between the velocity of the enzyme action and the concentration of the enzyme, by putting x equal to the reciprocal of the time taken to effect a given change, and y to the concentration of the enzyme. This interpretation of the simple adsorption equation seems to be incorrect, since y, in the adsorption equation, stands for the concentration of the substance still unadsorbed, while Bayliss puts y equal to the amount of adsorbent or enzyme. The only relation between Bayliss' equation and the adsorption equation is that both are of the parabolic type.

[1] *Proc. Roy. Soc. London*, (*B*) **84**, 90 (1911).

Bayliss found by applying his equation to the experimental data, that he obtained in the study of invertase action, that n had a value of 3.7. Other investigators, O'Sullivan and Tompson, Brown, Hudson, Michaelis and Davidson,[1] found that the velocity of inversion was proportional to the invertase concentration.

If the latter is true, then n is 1 in the above equation of Bayliss and the equation assumes the form $x = ky$, and since x is the reciprocal of the time necessary to effect a given change, t, or $1/x = t$, then the product of this time times the concentration of invertase is a constant, or ty = constant.

If the time necessary to effect a given change is proportional to the sugar concentration c, then ty/c = constant.

If the time necessary to effect a given change is independent of the sugar concentration, as required by the unimolecular law, then the value of ty will be independent of the sugar concentration.

The values for t, y and c can all be determined experimentally, and therefore it ought to be possible to ascertain whether any of the relationships enumerated in the preceding paragraphs exist. In Table III the results of the first 15 experiments have been so arranged as to bring out for comparison the values of ty and ty/c.

TABLE III.

1. No. of expt.	2. p_H+.	3. Gr. sugar per 100 cc. c.	4. Cc. invertase per 100 cc. y.	5. Time for 80% inversion. t.	6. ty.	7. ty/c.
1.....	4.5	5	6	90.5	543	109
2.....	4.5	5	5	109	545	109
3.....	4.5	5	4	136	544	109
4.....	4.45	5	3	179	537	107
5.....	4.45	5	2	279	558	112
6.....	4.58	10	6	182	1092	109
7.....	4.5	10	5	213	1065	107
8.....	4.58	10	4	264	1056	106
9.....	4.5	10	3	375	1125	113
10.....	4.58	10	2	545	1090	109
11.....	4.42	20	6	425	2550	128
12.....	4.45	20	5	511	2555	128
13.....	4.52	20	4	635	2540	127
14.....	4.5	20	3	856	2568	128
15.....	4.45	20	2	1338	2676	134

The time required for 80% inversion was chosen because it covers the greater part of the inversion and also because there is in each case a sample taken near that point so there would be little error in interpolating to find the exact time for 80% inversion. A graphic method of interpolation was used, the per cents. inverted being plotted as abscissas and the time as ordinates. By plotting the curves on a large scale, the time

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

for any given per cent. of inversion up to about 95% could be read quite accurately.

Invertase action is directly proportional to the concentration of the invertase. An examination of Col. 6 in Table III shows that if Bayliss' equation applies to invertase action, n is 1 since ty is constant for the same concentration of sugar, and not 3.7 as Bayliss found it to be. The velocity of inversion is therefore directly proportional to the invertase concentration as found by the investigators mentioned above, and not an "exponential function" of the enzyme concentration as claimed by Bayliss.[1]

The present authors are unable to acccount for the fact that Bayliss obtained the value of 3.7 for n, since he does not give very much detail in the description of his experiments.

The hydrogen-ion concentration does not influence the relationship between the velocity of inversion and the invertase concentration. The figures in Col. 6, Table IV, also show that ty is constant for the same sugar concentrations, or the velocity of inversion is directly proportional to the invertase concentration when the hydrogen-ion concentration is $10^{-5.5}$, instead of $10^{-4.5}$, as in the case of the experiments in Table III.

Change in concentration of cane sugar does not effect the relationship between the velocity of inversion and the concentration of invertase. This is evident from the values of ty, for the same concentrations of sugar, in Tables III and IV, where the cane sugar concentrations vary from 5 to 20 g. per 100 cc. Whether this relationship also holds for more dilute sugar solutions was not determined in this investigation. Michaelis and Menton, however, give values in their Tables 2, 3 and 4, for velocity of inversion and amount of invertase, for dilute sugar solutions, in which the sugar concentration is constant, and the relationship seems to hold. Too much reliance, however, cannot be placed on their results, since they used a polariscope for determining the extent of inversion, and for dilute sugar solutions this method is not very satisfactory, due to the experimental errors being so large.

It might also be pointed out, in this connection, that the relationship between the velocity of inversion and the amount of inversion holds at different stages of the reaction since in Table III the measurements were made at 80% and in Table IV at 40% inversion.

Relationship between Concentration of Cane Sugar and the Velocity of Inversion.—Col. 7 in Table III shows that ty/c is constant for the sugar concentrations of 5 and 10 g. per 100 cc. Therefore the time

[1] The objection might be raised against the results of the above-mentioned investigators, except those of Michaelis and Davidson, that their results might not be absolutely trustworthy due to no definite statements being given as to the hydrogen-ion concentrations. This objection is eliminated, however, in the present results.

required to invert a given amount, 80%, of sugar is directly proportional to the sugar concentration and not independent of it as required by the unimolecular law. In the case of 20 g. per 100 cc. the value of ty/c is larger, showing that the time required for 80% inversion is more than twice as great as the corresponding time when the sugar concentration is 10 g. per 100 cc. Brown also noticed a similar effect in the more concentrated sugar solutions. He found the time necessary for the same per cent. inversion in the case of 20 g. per 100 cc. was just twice that for 10 g. per 100 cc., but in the case of 40 g. per 100 cc. the time required was more than twice that in the case of 20 g. per 100 cc.

Col. 7 in Table IV shows that ty/c is approximately constant here too, and the velocity of inversion is nearly independent of the sugar concentration. It must be mentioned, however, that there is a slight difference between the values of ty/c in the two Tables, III and IV, in that the ty/c, Table IV, has a minimum value when the sugar concentration is in the neighborhood of 10 g. per 100 cc., or, in other words, the velocity of inversion reaches a maximum somewhere near this concentration, while in Table III no such maximum is apparent, but instead a slight decrease in the velocity, or increase for ty/c, in the more concentrated sugar solution, 20 g. per 100 cc. This difference might possibly be due to the solutions in the two tables having different hydrogen-ion concentrations and different preparations of invertase.

TABLE IV.

Relationship between the Time for 40% Inversion and the Concentrations of Cane Sugar and Invertase.

Expt. No.	p_{H+}.	G. sugar per 100 cc. c.	Cc. of invertase B. y.	Time for 40% inversion. t.	ty.	ty/c.
16.....	5.47	5	2	47.5	95	19.0
17.....	5.43	5	1	93	93	18.6
18.....	5.50	10	2	79	158	15.8
19.....	5.35	10	1	163	163	16.3
20.....	5.44	20	2	175	350	17.5
21.....	5.45	20	1	376	376	18.8

The data from Experiments 1–21, used in Tables III and IV, only deal with relatively concentrated sugar solutions. In order to see whether the same relationship, between velocity of inversion and concentration of sugar, also holds for more dilute sugar solutions, the results of Experiments 25–31 have been arranged in Table V, similarly to those in Tables III and IV.

In Experiments 25–31 the same hydrogen-ion concentration, $c_{H+} = 10^{-4.4}$, and invertase concentration were maintained throughout, and only the sugar concentration varied. This was done in order to keep as many factors, which might influence the velocity of inversion, constant as possible. The time required for 25% inversion was selected in each case,

since at this stage of the reaction the effect of the invert sugar is cut down to a minimum.

TABLE V.

Expt. No.	p_H+.	G. sugar per 100 cc. c.	Cc. dilute invertase A. y.	Time for 25% inversion. t.	ty/c.	c/t.
25.....	4.30	20	4	728	135.6	0.0275
26.....	4.30	10	4	309	123.6	0.0324
27.....	4.38	5	4	152	121.6	0.0329
28.....	4.30	4	4	126	126	0.0318
29.....	4.33	2	4	68	136	0.0294
30.....	4.36	1	4	48	192	0.0208
31.....	4.42	0.4	4	34	340	0.0085

It is to be noted that the values for ty/c in Table V are not nearly as constant as those for the more concentrated sugar solutions given in Tables III and IV. The inversion velocity as measured by c/t, a measure of the mean velocity, or weight of sugar inverted per unit time during the first quarter of the inversion is small and ty/c is large for the sugar concentration 0.4 g. per 100 cc., but the velocity increases as the sugar concentration increases and finally reaches a maximum value for c/t and a minimum value for ty/c between 5 and 10 g. per 100 cc. (Experiments 26 and 27).

In Fig. 2, the results of Experiments 25–31, as given in Table V, are plotted as Curve I in such a way that the ordinates are c/t, a quantity which is proportional to the mean velocity of inversion during the first 25% inversion. Curves II and III are similar curves plotted from the results of Michaelis and Menton, the ordinates for the latter curves being the initial velocities and the abscissas being the sugar concentrations as given in their Tables II and IV.

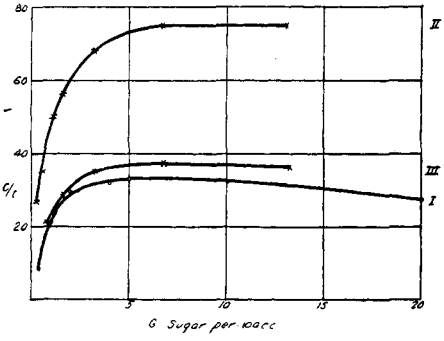

Fig. 2.

The velocity of inversion reaches a maximum as the concentration of sugar is increased. In each of the above curves, the line curves until the concentration reaches about 5 g. per 100 cc., after which it becomes parallel to the x axis, which means that the velocity is independent of the sugar concentration from 5 g. per 100 cc. up to very concentrated sugar solutions, in case of the experiments studied.

Application of Other Adsorption Equations to the Kinetics of Invertase Action.—Although it has been shown by the results indicated in the above tables, that the velocity of inversion is directly proportional to the enzyme concentration, no matter what particular concentration employed, and for different preparations of invertase, A and B, and consequently exclud-

ing the equation proposed by Bayliss, it by no means follows that adsorption is not one of the controlling factors in the kinetics of invertase action.

More recently, Bayliss,[1] due to the variation of the value of n in his equation above, at different stages of the reaction, has proposed a more general equation for the kinetics of enzyme action,

$$dc/dt = kc^n.$$

This equation differs from the first one in that n is not a constant but an unknown function of c, the concentration of the substrate or its products. It might, however, be said that this last expression is so general in character that it would apply to almost any kind of reaction.

There are, however, heterogeneous reactions of this type which apparently do follow a simple law like

$$dc/dt = kc^n,$$

which only differs from this last equation of Bayliss in that n instead of being a function of the concentration of the substrate, is a constant, equal to $^1/_2$ or some other value not 1 as in the case of unimolecular reactions.

The dissociation of stibine into antimony and hydrogen, in the presence of metallic antimony, studied by Stock and Bodenstein,[2] satisfies the above equation when n is $^1/_2$. It is therefore an example of this type. Since this reaction is similar to invertase action in that in both, the reactants, the stibine and the cane sugar, are adsorbed to the catalyst and then decomposed, it emphasizes the fact that the invertase action is of a more complicated nature.

Langmuir[3] also finds that the velocity of dissociation of molecular hydrogen into atomic hydrogen is proportional to the square root of the pressure of the molecular hydrogen. Langmuir discusses the kinetics of these reactions in the light of his theory of adsorption. He considers two classes of reactions. 1st. Reactions in which the adsorbed film covers only a small fraction of the surface of the adsorbent. 2nd. Reactions in which the surface is nearly completely covered by the adsorbed film. He assumes, in his general development, that the velocity of the reaction is not fast enough to materially effect the equilibrium between the adsorbed film and the surrounding gas. By means of the first class, he is able to account for reactions like the dissociation of molecular hydrogen, obeying the above law when n is $^1/_2$. He suggests the second of his two classes as applicable to enzyme action, but so far the present authors have not been able to apply, without further assumptions, his theory to invertase action. That there are two extreme classes of heterogeneous

[1] *Science*, **42**, 513 (1915).
[2] *Ber.*, 40, 570 (1907).
[3] THIS JOURNAL, **38**, 2289 (1916).

catalytic reactions of this type appears possible. However, it might be, not as Langmuir considers it, that the velocity of reaction is not fast enough to materially affect the equilibrium between the adsorbed film and the surrounding gas, but rather that the two classes are different due to the difference between the relative velocities of adsorption and reaction. Thus, in the first class, the velocity of reaction is greater than the rate of adsorption and thereby preventing the accumulation of any appreciable amount of substance or reactant on the adsorbent, while in the second class, the amount of reactant adsorbed per unit time is greater than the amount of reactant decomposed, thereby causing the absorbent to become saturated with respect to the reactant. In this way the second class would correspond to invertase action in the more concentrated cane sugar solutions, since in these solutions the invertase would be saturated with respect to the substrate, and the reaction velocity would then be directly proportional to the amount or concentration of invertase present as shown by the results obtained in this investigation. The maximum in reaction velocity, reached in passing from dilute sugar solutions to concentrated, is that point where the amount of cane sugar adsorbed per unit time is just equal to the maximum rate of hydrolysis. Below the maximum point, the velocity of inversion will be dependent both upon the concentrations of sugar and invertase.

The Mean Velocity of Inversion, a Measure of the Amount of Sugar Adsorbed.—Being heterogeneous in character, it follows that the inversion must take place on or near the surface of the invertase, and that the concentration of cane sugar present at the surface will be the active sugar concentration. Furthermore, the active concentration of sugar and therefore the mean velocity of inversion up to 25% inversion, c/t, might be considered as a measure of the amount of cane sugar adsorbed by the invertase. Under this assumption, the Curves I, II and III in Fig. 2 become curves which show the relative amounts of cane sugar adsorbed by the invertase in the sugar solutions of different concentrations of sugar. The fact that there is a maximum mean velocity of inversion, a maximum value for c/t at 5 and 10 grams of sugar per 100 cc. solution and beyond this the curves become parallel to the x axis, indicates at this concentration of sugar there is a maximum amount of cane sugar adsorbed.

It is very interesting to note that the shape of Curves I, II and III in Fig. 2 is the same as that of the adsorption curve which Schmidt[1] found in studying the adsorption of acetic acid by charcoal. Schmidt found that the charcoal had a definite maximum capacity for acetic acid and that when this limit was reached no more was adsorbed even though the concentration of acetic acid was greatly increased. This saturation

[1] *Z. physik. Chem.*, **74**, 699 (1910).

effect is not taken into account in the ordinary adsorption equation of Freundlich, $x/m = kc^{1/n}$, and so Schmidt proposed the equation

$$\log (S/(S - a)) - Aa = 1/kc.$$

S is the maximum amount of adsorption, a is the amount of substance adsorbed, and c the original concentration of the substance being adsorbed. A, k and S are constants. The equation has, however, not proved satisfactory and Schmidt has suggested other equations[1] which also seem to have some objections.

Arrhenius[2] claims that Schmidt's experimental results indicate that A is a function of S, and that the product of the two, AS, is a constant equal to 0.4343, the factor for converting natural logarithms into logarithms to the base ten. Arrhenius points out that due to this fact it is possible to reduce Schmidt's equation to the simple differential equation

$$da/dc = 1/k((S - a)/a).$$

Upon integrating, this becomes

$$k = S/c \log (S/(S - a)) - a/c.$$

It has already been pointed out that c/t can be regarded as a measure of the amount of sugar adsorbed and therefore would correspond to the value of a in the above equation of Arrhenius, and the maximum value of c/t, 0.0327, would be S, the maximum amount of sugar adsorbed. On applying the results, obtained in this way from Experiments 28–31, to the above equation, the values for k in Table VI are obtained. It is seen that k is far from being constant, and therefore it is evident that this equation of Arrhenius is not applicable to the kinetics of invertase action.

TABLE VI.

Expt. No.	28.	29.	30.	31.
c	4	2	1	0.4
a	0.0318	0.0294	0.0208	0.0085
k	0.0583	0.0228	0.0184	—0.0048

S was taken as 0.0327 from Experiments 26 and 27, Table V.

Preparation of the Invertase.

The invertase used in Experiments 1–15 and designated as Invertase A, was prepared by the method of Nelson and Born.[3] One precipitation of the invertase by alcohol was omitted and the invertase solution was dialyzed in running water for four days before the final precipitation. A solution was made containing 10 g. of the dry preparation per liter and various portions of this solution used as described.

The invertase used in Experiments 16–23 and designated as Invertase B was prepared without the use of alcohol. The liquid resulting from the autolysis of the yeast was filtered, the filtrate treated with lead acetate

[1] *Z. physik. Chem.*, **77**, 646 (1911).

[2] "Theories of Solutions," Yale Univ. Press, 1912, p. 61.

[3] THIS JOURNAL, **36**, 393 (1914).

and the precipitated proteins, etc., filtered off. The lead was then removed by the addition of saturated potassium oxalate solution and the filtrate dialyzed in collodion bags in running water for four days. Invertase B was not obtained in the dry state but portions of the dialyzed solution were used for Experiments 16–23.

The invertase solution used in Experiments 25–31, designated as Dilute Invertase A, was prepared by diluting a portion of the above described Invertase A solution, four times its volume.

To show that the activity of Invertase A did not change while the investigation was in progress, the determination of activity A was carried out at the beginning, and the determination B at the end of the series of experiments in the investigation. The agreement between the two shows that the activity remained the same. Each solution contained 10 g. of cane sugar and 2 cc. of invertase, and in the case of A, 0.54 cc. and in the case of B, 0.4 cc. of 0.01 molar hydrochloric acid per 100 cc. solution. The hydrogen-ion concentration for A was $p_{H^+} = 4.58$, and for B, $p_{H^+} = 4.6$.

Time (minutes)	0	50	100	221
Rotation—A	12.00°	10.45°	8.87°	5.47°
Rotation—B	12.04°	10.44°	8.88°	5.53°

A peculiar phenomenon with regard to the dilution of invertase solution was noticed, and it is hoped that a further investigation of this question can be reported in the future. Under certain conditions, the invertase solutions could be diluted without changing their activity. For example, when Invertase A solution used in Experiments 1–15 was diluted with distilled water, containing a small amount of hydrochloric acid, in order to obtain approximately the desired acidity, no trouble was experienced in obtaining duplicates. When, however, the acid was omitted, then solutions were obtained whose activity differed widely. In making up Dilute Invertase A solution, although the original solution was diluted to four times its volume, the activity of the resulting solution was less than one-fourth that of the original. Some preliminary experiments showed that the loss of activity on dilution was a characteristic of some invertase preparations but not of all. The authors believe the experiments reported in this paper to be free from this error.

Procedure.

The procedure for Experiments 1–24 was the following: The required amount of invertase solution was measured out and diluted with water, the necessary amount of 0.01 molar hydrochloric acid added and the solution made up to 250 cc. It was then tested as to hydrogen-ion content as described below and brought to the desired concentration by the addition of a small amount of 0.1 or 0.01 molar hydrochloric acid.

Two hundred cc. of this solution were then measured out and placed in a thermostat at 37° ± 0.02. Two hundred cc. of a sugar solution, twice as strong as that desired for the inversion, were placed in the bath at the same time and after allowing 30 minutes for the solutions to come to the temperature of the bath, the two were mixed. A sample was taken as soon after mixing as possible, usually one or two minutes, and another after an interval equal to twice that at which the first was taken, and the angular rotation of each measured. In this way it could be found out how much the rotation had changed in the second small time interval and this was assumed equal to the change in the first, and the initial angle calculated. Samples were then taken at the times at which the solutions would be approximately 10, 20, 40, 60, 80, 95 and 100% inverted, and the rotation of each measured. The sugar was considered to be 100% inverted when samples taken on two successive days gave the same angular rotation.

Errors due to the mutarotation of the invert sugar were eliminated by adding the 20 cc. sample to 2 cc. of 0.2 molar sodium carbonate solution at the time t as recommended by Hudson.

In Experiments 25–31, 400 cc. of sugar solution, containing such an amount of sugar that when diluted to 500 cc. it had the desired concentration, were measured out. About 1.5 cc. of 0.02 molar hydrochloric acid and 78.5 cc. of distilled water were added and the solution placed in the thermostat. At the time t = 0, 20 cc. of dilute invertase A, previously heated to 37° in the thermostat, were added and the solution well mixed.

Determination of the Amount of Inversion.

All measurements of rotation were made by means of a polariscope using a 200 mm. water-jacketed tube which was kept at constant temperature by pumping water through it from a water thermostat regulated at 25° ± 0.01. The zero point of the polariscope was checked each day. Each reading is the mean of at least four concordant readings.

In Experiments 28–31, on account of the small concentration of the sugar in these experiments, it was necessary to use the modification of Defren's method as described by Fales and Nelson. Samples were taken, of such volume that they contained the equivalent of 0.2 g. of cane sugar, instead of 0.05 g. as in the determinations of Fales and Nelson, and were run into one-tenth of their volume of 0.2 molar sodium carbonate solution to stop the action of the invertase, and the extent of the inversion determined. Since the samples contained 0.2 g. of sugar, the sugar table of Fales and Nelson could not be used. For the purpose of calculating the per cent. inverted from the amount of copper oxide obtained, Experiments 25 and 26, containing 20 and 10 g. of sugar per 100 cc., were carried out, using both the polariscope method and the copper reduction

methods. Fifty cc. samples were taken and run into 5 cc. of 0.2 molar sodium carbonate solution. Two 5 cc. samples of the resulting solution were carefully measured out and diluted to 100 cc. with a dilute sodium carbonate solution. In Experiments 25, a 22 cc. portion of this solution was used for the copper reduction method, and in Experiment 26 a 44 cc. portion was used, these volumes each containing 0.2 g. of sugar. The remaining portions of the original 55 cc. were used for determination of the rotation. The results are given in Table I. The amounts of cupric oxide were plotted as ordinates and the per cents. inverted as abscissas, giving a curve which represents the relation between the per cent. inverted as determined by the polariscope and the weight of copper oxide obtained when a 0.2 g. sample was used. The per cents. given in Experiments 28–31 were read from this curve. The figures in parentheses are values that lay beyond the end of the curve (which was nearly a straight line) and hence were extrapolated. These points are all beyond 25% inversion and hence are not important.

Control of the Hydrogen-Ion Concentration.

The hydrogen-ion concentration of all the solutions was measured during the early part of the inversion by the colorimetric method.

Sorensen has shown that it is necessary in working with invertase to control the hydrogen-ion concentration and he recommends the use of buffers for this purpose, since two solutions made up in the same way but without a buffer, may vary enough to cause a measurable difference in the rate of inversion. It has been shown by Fales and Nelson that sodium chloride has a retarding effect on the velocity of inversion, and although the effect is small at the hydrogen-ion concentration used in this investigation, it was better to eliminate the little known salt effect of the buffers and to add only enough hydrochloric acid to bring the solution to the required hydrogen-ion concentration.

The regulation of the hydrogen-ion concentration was carried out in the following manner: Enough invertase to make 500 cc. of the desired solution was diluted with water containing nearly enough hydrochloric acid to produce the required acidity, and the solution made up to 250 cc. For a preliminary test of the acidity, 5 cc. were measured out and added to 5 cc. of a sugar solution twice as strong as desired for the inversion and the hydrogen-ion concentration measured colorimetrically. Small portions of 0.1 or 0.01 molar hydrochloric acid were added until the solution, tested as above, was shown to contain the correct concentration of hydrogen ion. In no case over 0.7 cc. of acid was added after making up the solution of invertase and acid so no serious error was introduced due to incorrect volume. After the hydrogen-ion concentration was thus adjusted, 200 cc. of diluted and acidified invertase and 200

cc. of sugar solution of twice the desired strength were placed in the bath for 30 minutes and then mixed.

For the measurement of the hydrogen-ion concentration the electromotive force method is not applicable. In some preliminary experiments it was tried but concordant results were not obtained. The method using bubbling hydrogen is not applicable on account of the high resistance of the solution to be measured and consequently low sensibility of the system. By using a Clarke electrode[1] and still hydrogen the column of liquid between the electrode and the salt bridge is much shorter than in the above type of vessel and the high resistance did not have so much effect. It was found, however, that the hydrogen-ion concentration increased more or less when the solution to be measured was left in contact with hydrogen and platinum (but not with hydrogen alone) so obviously the method could not be used.

The colorimetric method described by Sorensen was therefore employed. Standard hydrogen-ion solutions were made up by using mixtures of secondary sodium citrate and hydrochloric acid or sodium hydroxide and naphthylaminoazo-*p*-benzene sulfonic acid or *p*-nitrophenol for indicators, whose hydrogen-ion concentrations were near that of the solution to be measured and differed from each other by 0.1 in the exponent of 10. Thus for all experiments in which the hydrogen-ion concentration was $10^{-4.1}$, standards having concentrations $10^{-4.2}$, $10^{-4.3}$, $10^{-4.4}$, $10^{-4.5}$ and $10^{-4.6}$ were prepared and used as described by Sorensen. The hydrogen-ion concentration of each standard was determined by the electromotive force method, using the system Hg–HgCl, 0.1 *N* KCl–3.5 *N* KCl–unknown–H_2Pt. The calomel electrode was standardized by measuring the voltage given when a 0.1 *M* hydrochloric acid solution was substituted for the unknown.

Fales and Nelson concluded that the hydrogen-ion concentration does not change throughout the inversion. Several tests were made at the end as well as near the beginning of the inversions in Experiments 1–15 and 25–31, which showed that in solutions such as these no change can be noticed during the inversion.

In some preliminary experiments using Invertase A at the hydrogen-ion concentration $10^{-6.2}$ and $10^{-5.5}$ it was found that the hydrogen-ion concentration increased slightly during the inversion, and the results of the inversions were not in satisfactory agreement with each other. When Invertase B was tried at the latter hydrogen-ion concentration, $10^{-5.5}$, it was found that it also changed slightly although the change was not noticeable until the sugar was 50% inverted. Therefore the results of Experiments 16–21 are accurate, as far as acidity is concerned, at least up to 50% inversion.

[1] *J. Biol. Chem.*, **23**, 475 (1915).

The hydrogen-ion concentrations in Experiments 1–15 lie between $10^{-4.42}$ and $10^{-4.58}$. The experiment given below gives an idea of the effect of a small variation in the acidity. This experiment is a duplicate of Experiment 13, Table I. The velocity coefficients of Experiment 13 ($C_{H^+} = 10^{-4.52}$) are given in the last column for comparison. A comparison of the two shows that the difference in hydrogen-ion concentration makes little difference in the velocity of the reaction.

20 g. Sugar and 2 cc. Invertase A per 100 cc. $C_{H^+} = 10^{-4.38}$.

Time.	Observed rotation.	Per cent. inversion.	$1/t \log 1/1-x'$.	Vel. coeff. Expt. No. 13.
0	24.15	.....	.....	.....
55	21.07	9.82	0.00082	0.00081
115	17.88	19.99	0.00084	0.00086
240	11.69	39.72	0.00092	0.00093
359	6.69	55.66	0.00098	0.00101
710	—2.01	83.39	0.00110	0.00110
1340	—6.00	96.11	0.00105	0.00104
	—7.22	.....	.....	.....

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MONTANA.]

COPPER IN THE FLORA OF A COPPER-TAILING REGION.

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During the past decade extensive and valuable information has contributed much to the knowledge of the effect of smelter smoke and dust on the plants and stock living in adjacent regions. Another waste product of the smelters, the tailings, has, however, received little attention. This paper notes some effects caused by the tailings of a large copper smelter on the native flora of the vicinity.

This smelter is that located near Anaconda, Montana. It is situated at the base of a mountain ridge at the Southwest border of Deer Lodge Valley, which is about forty miles long and from four to six miles wide. The tailings from the smelter are emptied into Warm Springs Creek, find their way across the valley in many small streams and ultimately flow into the Missoula River. Along the way the heavier metallic particles of the material are gradually deposited while many other streams join the main waterway of the region. But even at Missoula, a distance of about one hundred miles, the water is very turbid from the tailings of the smelter above.

A trip through the region affected by the tailings presents a very interesting picture. Before their advent the soil supported the characteristic flora of this district which is still seen outside the tailing areas and across the river on the other side of the valley. There flourishing willows line the little streams while grasses of various kinds, the wild rose, and clover