for small green leaves at the top. The controls, I_{1-3} and $VIII_{1-3}$ were in fairly good condition.

Feb. 18. $III_{1,2}$ completely withered.

Feb. 21. $I_{2,3}$ not quite so healthy; IV_{1-3} and V_1 nearly dried up. I_1 and $VIII_{2,3}$ growing well.

Feb. 26. I_3 dying, III_3, $IV_{2,3}$, $V_{1,2}$, VI_{1-3}, VII_{1-3}, in bad condition.

On the last day of observation, March 3, practically all the cultures in glucosamine solutions were completely withered; none were growing well. Of the controls in solution I, one was withered and two were growing well. Of the controls without nitrogen, solution VIII, none was growing normally.

Conclusions.

From the foregoing experiments it is evident that under the conditions of growth glucosamine could not be utilized as a source of nitrogen for nutrition, owing either directly to its own characteristics, or indirectly, to conditions it may have caused, such as the growth of some mould.

[CONTRIBUTIONS FROM THE LABORATORIES OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS AND OF JEFFERSON MEDICAL COLLEGE.]

INHIBITION OF ENZYME ACTION BY LIME-SOFTENED WATERS.

BY OLAF BERGEIM AND P. B. HAWK.

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In the course of a study of the effects of dilution, with various waters, on the rapidity of digestion of starch by salivary amylase,[1] it was noted that the lime-softened waters used exerted a pronounced inhibitory influence on the action of this enzyme. The source of the water used was the University of Illinois water supply. As this is quite representative of a large class of hard well waters, which require treatment to render them palatable, it was considered of interest to determin the nature of the substances left by the lime treatment which produced the above noted effects.

The water before treatment contained[2] 60.5 parts Na_2CO_3, 121.2 parts $MgCO_3$ and 175.2 parts of $CaCO_3$ per million, with small amounts of other salts. It was softened by the addition of one-fifth its volume of saturated lime water, and then filtered. Two specimens of this softened water were used in the tests; the first designated as (3–11) had remained loosely stoppered for several months and the second designated as (9–11) was prepared just before beginning the experiments.

A series of tests illustrating the inhibiting action of these waters on amylolytic activity is outlined in Table I. Saliva and a solution of Armour's "Amylopsin" were used as digestive agents and Kahlbaum's soluble starch was employed as the substrate. The time for the digestion mixture to reach the achromic point with iodine was measured in each case.

[1] Bergeim and Hawk, THIS JOURNAL, **35**, 461 (1913).

[2] Bartow and Lindgren, U. of Ill. State Water Survey, *Bull.* 6, 1908.

TABLE I.

Temperature 20°.

				Time to the achromic point.
1	50 cc. 2% starch	50 cc. 0.3% NaCl sol.	5 cc. amylop. sol.	1.33 hours
2	"	50 cc. dist. water	"	2.80 "
3	"	50 cc. tap water	"	12.00 "
4	"	50 cc. soft (3–11)	"	12.00 "
5	"	50 cc. soft (9–11)	"	15.00 "
6	"	50 cc. 0.3% NaCl sol.	1/10 cc. saliva	1.80 "
7	"	50 cc. dist. water	"	4.33 "
8	"	50 cc. tap water	"	13.00 "
9	"	50 cc. soft (3–11)	"	15.00 "
10	"	50 cc. soft (9–11)	"	22.00 "

From this table it will be noted that the action of both salivary and pancreatic amylase was pronouncedly inhibited, the inhibition being greatest with the recently softened water and least with the tap water. Distilled water was a more satisfactory diluent, whereas the sodium chloride solution was much more satisfactory than any of the waters mentioned. Pancreatic amylase was somewhat less affected by the inhibiting agents than the salivary amylase.

It seemed quite possible that this inhibiting action of the waters softened with lime might be due to their greater alkalinity. Therefore, both of these softened waters as well as tap water were titrated, first with methyl orange as an indicator to obtain the total amount of hydroxides and carbonates present, and second using phenolphthalein with boiling and cooling of the solution, which gave the total hydroxides with one-half of the alkali carbonates, not including any of the carbonates of the alkaline earths.

The results were as follows:

1. Total hydroxides and carbonates.
   Indicator: Methyl orange.
   100 cc. tap water required 3.3 cc. $N/5$ HCl.
   100 cc. soft water (3–11) required 1.8 cc. $N/5$ HCl.
   100 cc. soft water (9–11) required 1.8 cc. $N/5$ HCl.
2. Total hydroxides and one-half of alkali carbonates.
   Indicator: Phenolphthalein, with boiling and cooling.
   100 cc. tap water required 10.05 cc. $N/5$ HCl.
   100 cc. soft water (3–11) required 6.75 cc. $N/5$ HCl.
   100 cc. soft water (9–11) required 6.70 cc. $N/5$ HCl.

It will be noted that the alkalinities of the two soft waters were practically the same while that of tap water was somewhat greater. It appeared, therefore, that the differences of action could not be accounted for merely on the basis of alkalinity.

A calcium test was made to determin whether an excess of lime had been added in the softening process. Only a slight test was obtained, however, such as might be accounted for by the presence of small amounts of cal-

cium carbonate. A much more pronounced test was obtained with tap water.

In order to learn the composition of the softened water and to determin, if possible, the nature of the inhibiting factor, Prof, E. Bartow, Director of the Illinois Water Survey, was consulted. From data submitted on the treatment of the University water supply with lime,[1] it was determined that the following was the probable composition of the water used in our tests:

Sodium carbonate	60.5 parts per million.
Calcium carbonate (saturated solution)	30–50 parts per million.
Magnesium hydroxide (saturated solution)	15–20 parts per million.

To determin the action of these substances, separately and mixed, on the enzyme hydrolysis of starch, the following solutions were made up:

Saturated solution of magnesium hydroxide (freshly made).

Saturated solution of calcium carbonate.

Solution of sodium carbonate, 60 parts per million.

Saturated solution of magnesium hydroxide and calcium carbonate in sodium carbonate solution, 60 parts per million.

To determin whether these ingredients, separately or combined, were sufficient to account for the inhibiting action of the softened waters several series of quantitative tests were made. The first two series were run, using the salivary enzyme, and the second two using Armour's preparation of amylopsin. The reducing sugar was determined by the reduction of Fehling's solution[2] filtering off the resulting cuprous oxide, dissolving in nitric acid and titrating according to Kendall's modification of the copper-iodide method.[3] Representative results are given in the following tables:

TABLE II.—INHIBITION OF THE ACTION OF SALIVARY AMYLASE BY CERTAIN SUBSTANCES CONTAINED IN SOFTENED WATER.

Time of Digestion: 1 hour. Temperature: 26°.

No.	Kind of water.	No. cc. water.	No. cc. of 4 per cent. starch.	No. cc. saliva.	No. cc. of $Na_2S_2O_3$.	No. mg. of maltose.
1	Distilled	100	25	0.2	27.76	111.04
2	$CaCO_3$ sat. sol.	"	"	"	16.71	66.84
3	$Mg(OH)_2$ sat. sol.	"	"	"	0.47	1.88
4	Na_2CO_3 60 p. m.	"	"	"	10.80	43.20
5	Artificial soft[2]	"	"	"	0.02	0.08
6	Tap water	"	"	"	8.96	35.84
7	Soft (3–11)	"	"	"	4.31	17.24
8	Soft (9–11)	"	"	"	0.86	3.44
9	Check	"	"	"	6.49	....

All columns corrected for check test.

[1] Bartow and Lindgren, U. of Ill. State Water Survey, *Bull.* 6, 1908.

[2] The artificial softened water is the before mentioned mixture containing 60 parts per million of Na_2CO_3 and being saturated with $CaCO_3$ and $Mg(OH)_2$.

[3] THIS JOURNAL, 33, 1947 (1911); 34, 317 (1912).

TABLE III.—ON THE INHIBITING ACTION OF SUBSTANCES CONTAINED IN SOFT WATERS.
Enzyme used: Pancreatic amylase. Time: 2 Hours. Temperature: 38°.

No.	Kind of water.	No. cc. water.	No. cc. of 4 per cent. starch.	Pancreatic amylase. Mg.	No. cc. of $Na_2S_2O_3$.	No. mg. maltose.
1	0.3% NaCl sol.	100	25	2.5	57.71	230.84
2	Distilled	"	"	"	14.55	58.20
3	$CaCO_3$ sat. sol.	"	"	"	17.45	69.80
4	$Mg(OH)_2$ sat. sol.	"	"	"	0.60	2.40
5	Na_2CO_3 60 p. m.	"	"	"	15.35	61.40
6	Artificial soft[1]	"	"	"	0.55	2.20
7	Tap water	"	"	"	11.00	44.00
8	Soft (3–11)	"	"	"	5.55	22.20
9	Soft (9–11)	"	"	"	1.55	6.20
10	Check	"	"	...	7.50	....
11	Check	"	"	...	7.80	....

All columns corrected for check tests.

Discussion.

Under the conditions of these experiments, any one of the ingredients of softened water caused an inhibition of the action of salivary amylase as compared with distilled water, the order of inhibition being: 1, $Mg(OH)_2$ saturated solution; 2, Na_2CO_3 solution; 3, $CaCO_3$ saturated solution. The magnesium hydroxide exerted by far the greatest inhibition, the amount of reducing sugar produced in its presence either alone or in mixtures, being almost negligible. In the salivary digestions calcium carbonate exerted a very noticeable inhibitory action. Sodium carbonate solution still further decreased the activity of the enzyme. The softened waters were not quite as detrimental in their action as the artificial mixture, but the more recently softened water approached it.

All of these experiments pointed to the conclusion that the magnesium hydroxide was the principal inhibiting substance contained in these softened waters, although in the case of saliva, sodium carbonate also gave an unfavorable reaction. As to the degree of this inhibition, it will be seen that under the conditions of these experiments the magnesium hydroxide content reduced the enzyme activity to about 1/100 of its value in a 0.3% salt solution.

It was of interest to know whether a like inhibition was exerted by the magnesium hydroxide solution and the softened water toward the enzyme pepsin. To determin this and at the same time to find out whether or not this inhibition was due to the Mg ion, the series of tests outlined below were carried out, different amounts of these solutions being added to pepsin-HCl digestion mixtures[2] and the extent of the digestion, as measured by the Mett method,[3] taken as an index of the activity of the enzyme

[1] See note, Table II.

[2] Pepsin in 0.2% HCl.

[3] Inaug. Diss., St. Petersburg, 1889.

under the different conditions. Of course we are determining here the action, not of the magnesium hydroxide, but of corresponding amounts of magnesium chloride. The data are tabulated in Table IV.

TABLE IV.—PEPTIC DIGESTION IN THE PRESENCE OF MAGNESIUM CHLORIDE.

No.	No. cc. of pepsin·HCl.	No. cc. of dist. water.	Water tested.	Mm. albumin.[1] Average.	Activity.
1	10	10	.....	1.53	2.36
2	"	"	.....		
3	"	5	5 cc. $Mg(OH)_2$ sat.	1.60	2.56
4	"	"	"		
5	"	..	10 cc. "	1.53	2.36
6	"	..	"		
7	"	8	2 cc. "	1.60	2.56
8	"	"	"		
9	"	5	5 cc. soft (9–11)	1.60	2.56
10	"	"	"		
11	"	..	10 cc. "	1.40	1.96
12	"	..	"		
13	10 cc. 0.2% HCl	10	.....	0.00	0.00

Digestions carried out 36 hours at 40°.

In contrast to their inhibition of the action of the amylolytic ferments, we find here in the case of peptic digestion that the magnesium hydroxide solution and the softened water have very little influence. In the case where 10 cc. of the softened water was added the action was not quite as strong, but the difference is most probably a result of the decrease in acidity of the mixture due to the sodium carbonate of the softened water.

As the magnesium ion seemed to have no strong inhibiting action and as the alkalinity of the solution was not sufficient to account for the delay which the magnesium hydroxide caused in starch hydrolysis, the most plausible explanation of this inhibition was that the enzyme had been adsorbed by the colloidal magnesium hydroxide which had not been removed by the filtration. This hypothesis was tested out as follows: To each of six Erlenmeyer flasks was added 25 cc. of 2% starch paste. Then to the first of these was added 50 cc. of a saturated solution of $Mg(OH)_2$ prepared by dialyzing $Mg(OH)_2$, through a parchment membrane for ten days against distilled water. This should contain no colloidal $Mg(OH)_2$. To the second was added 50 cc. of $Mg(OH)_2$ solution, which had been filtered slowly through a foot of sand. To the third was added 50 cc. of distilled water, and to the fourth and fifth equal amounts of $Mg(OH)_2$ solution which had been filtered, respectively through hardened filter paper and through common filter paper. Finally in the sixth was placed unfiltered $Mg(OH)_2$ solution. Then to each flask 0.2 cc. of saliva was added and the time to the achromic point observed. The time of

[1] Figures are averages for four Mett tubes in each case.

digestion increased directly in the order given above, that is, the achromic point was reached first in the case where the dialyzed solution containing no colloidal $Mg(OH)_2$ was used, and last in the unfiltered $Mg(OH)_2$ solution, which contained the greatest amount of this material. Of the filters used, the sand was the most efficient in removing the inhibiting agent, while the hardened paper was less efficient and the common filter paper least efficient of all. It was also of interest to note that there was somewhat better digestion in the presence of the dialyzed solution than in the presence of the distilled water, indicating that the small amount of $Mg(OH)_2$ in true solution had no inhibiting action.

Similar combinations of enzymes with metallic hydroxides, kaolin and other colloidal materials have been studied by Michaelis and his coworkers.[1] They found that salivary amylase was adsorbed by kaolin, charcoal, and aluminium hydroxide, regardless of the reaction of the solution. They believe that the phenomena are in most cases related to the electrical character of the substances entering into combination.

Negatively charged colloids adsorb basic substances, while positively charged colloids adsorb only those acidic in character. The extent to which amphoteric substances are adsorbed depends on the reaction of the solution. Salivary amylase belongs to the latter class. Hedin[2] has made extensive studies of the adsorption of enzymes, particularly by charcoal.

As to the physiological effects of continuous use of such lime softened water we can say nothing definitely. There can, however, be little question that as far as salivary digestion is concerned, its action must be less favorable than that of a distilled water or one having a favorable electrolyte concentration.

Our work, as well as that of Kudo[3] indicates that the small amounts of $MgCl_2$ formed from the $Mg(OH)_2$ of these waters would have no noticeable effect on digestive processes. If, however, as has been noted in some cases, the water passes through the stomach without coming in contact with the gastric juice there might be some inhibition of intestinal digestion.

It would seem desirable that more emphasis be laid, in deciding as to the desirability of any drinking water, upon its influence on the digestive enzymes. From the standpoint of the highest efficiency of the organism we can hardly agree with such views as those of Dr. Ide,[4] who concludes

[1] Michaelis, Dynamik der Oberflächenwirkungen, Leipsic, 1909; *Biochem. Z.*, 7, 488 (1907); 12, 26 (1908); Michaelis and Ehrenreich, *Biochem. Z.*, 10, 283 (1908); Michaelis and Rona, *Biochem. Z.*, 15, 217 (1908).

[2] *Ergeb. die Physiol.*, 9, 433 (1910).

[3] *Biochem. Z.*, 15, 473 (1909).

[4] IIe Congrès internat. d'hygiene alimentaire et de l'alimentation rationelle de l'homme, Bruxelles, 1910.

that the influence of the character of the liquid ingested is negligible because compensation is provoked.

With regard to the lime softened waters it would appear that thorough sand or other filtration would eliminate the undesirable features noted, The same might be expected of the carbon dioxide treatment, with which the softening is sometimes supplemented.

Another point, however, should also be considered in this connection. Berg[1] and Rose[2] have endeavored to show, by comprehensive statistical studies, that there is a parallelism between the hardness of the water drunk by individuals, particularly in youth, and chest measurements, height, dental and bone diseases. As far as these conclusions have significance, they would indicate that hard waters possess certain advantages, due to their calcium content.

Our tests also throw some light on the question of the identity of the animal amylases. It will be noted from the tables that the calcium carbonate solution was more favorable than distilled water for the action of the pancreatic amylase while the reverse was found for salivary amylase. The same is true of the sodium carbonate solution, and tap water also exerted less inhibition on the pancreatic amylase. A like tendency is shown throughout, the salivary amylase being apparently also more readily adsorbed by the colloidal hydroxide. This latter fact would be explained by a greater negativity of the salivary amylase. Bierry, Henri and Schaffer[3] found that of several enzymes tested, including malt amylase, only pancreatic amylase traveled through a solution in the direction of the cathode. Welker and Marshall,[4] in a recent study of the precipitation of enzymes by moist aluminium hydroxide, have pointed out that of twelve enzymes tested, salivary amylase only was incompletely precipitated by this colloid. Pancreatic amylase was completely precipitated. It should be borne in mind that the pancreatic amylase used in our tests was not of human origin.

These indications as to the individuality of the two enzymes are not necessarily opposed to the findings of Musculus and v. Mering[5] and the confirmatory work of Külz and Vogel,[6] these authors showing that the same products are obtained in both salivary and pancreatic digestion. Nasse[7] based his conclusions, as to their identity, on the similarity of their action with relation to sodium sulfate, sodium nitrate, ammonium and

[1] *Biochem. Z.*, **24**, 282 (1910); **26**, 204 (1910).
[2] *Deutsche Monatsschr. f. Zahnheilk.*, 1904–1908.
[3] *Soc. Biol.*, **63**, 226 (1907).
[4] THIS JOURNAL, **35**, 822 (1913).
[5] *Bull. soc. chim.*, **31**, 105 (1879).
[6] *Z. Biol.*, **31**, 106 (1895).
[7] *Pflüg. Arch.*, **14**, 473 (1877).

potassium chlorides, and other salts, while Wohlgemuth[1] supports this view with results from the study of some neutral salts, acids, amino acids and bases. On the other hand, Vernon[2] has shown that salivary amylase acts much more slowly in the first few minutes of digestion than pancreatic amylase and points out differences in the action of several animal as well as plant amylases, Of course if the two enzymes are identical they must act similarly under all conditions, and apparent differences in their action constitute stronger evidence than any observed similarities and until these differences are satisfactorily accounted for on some other basis, we can hardly do otherwise than consider the enzymes distinct.

Conclusions.

Water softened by the use of lime was found to exert a pronounced inhibitory influence on the action of salivary and pancreatic amylases. This was due largely to the adsorption of the enzymes by colloidal magnesium hydroxide present in these softened waters.

That the two enzymes used in these tests, salivary and pancreatic amylases, are not identical, is indicated by differences shown in their response to the action of various ingredients contained in hard and softened waters.

[FROM THE LABORATORY OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN.]

A SIMPLE METHOD FOR THE DETERMINATION OF CARBON IN ORGANIC MATERIALS.

BY E. B. HART AND K. J. WOO.

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The determination of carbon in organic materials is still either a laborious process or involves the use of expensive apparatus. Its determination by the Liebig or old combustion method is at present standard, but only in research or in special courses of instruction is it used. The more rapid method of carbon determination devised by Parr[3] requires the use of a bomb and the liberation of the gas under standard conditions for volumetric measurement. So far as we know this method has only been applied to carbon determinations in coal and soil.[4] This method requires special apparatus which is somewhat expensive, although it gives accurate results.

The moist combustion methods which have been proposed for the determination of carbon in soils give low results. Incomplete oxidation

[1] *Biochem. Z.*, 9, 1 (1908).
[2] *J. Physiol.*, 28, 156 (1902).
[3] THIS JOURNAL, 26, 294 (1904).
[4] Pettit, *Ibid.*, 26, 1640 (1904).