Ethylbenzene.—Twenty-five g. of ethylbenzene and 25 g. of acetic anhydride oxidized for eleven days at 102–104° yielded 9.0 g. of crude acetophenone; previously reported, 9.5 g. of acetophenone from 50 g. of ethylbenzene in twenty-four days at 110–115°.

Xylyl Acetate.—Twelve g. of xylyl acetate, b. p. 103–106° (10 mm.), oxidized for eleven days at 102–104°, yielded no toluic aldehyde.

B. Parallel Oxidation of Ethylbenzene and Phenylmethyl Carbinol

Twenty-five g. of phenylmethyl carbinol, oxidized for eleven days at 122–124° yielded 0.85 g. of acetophenone, isolated as semicarbazone.

Twenty-one g. of ethylbenzene, oxidized as above for eleven days, yielded 6.3 g. of crude acetophenone, b. p. 77–83° (8 mm.).

Summary

A new mechanism of the oxidation of saturated hydrocarbons by gaseous oxygen is proposed, which accounts for the inhibitory action of water in oxidations studied by the writer.

It is shown that the oxidation of a hydrocarbon to aldehyde or ketone does not go through the alcohol stage.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, No. 578]

THE INTERRELATION OF HYDROGEN-ION ACTIVITY AND CONCENTRATION OF SALT IN THE ACTIVATION OF PANCREATIC AMYLASE[1]

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It has long been known that the presence of electrolyte is essential to the activity of pancreatic amylase, but quantitative data obtained under sufficiently controlled conditions have not been available to afford satisfactory explanations of this influence or to permit strictly quantitative comparisons of different electrolytes.

In view of this fact and of the theoretical and practical importance of the subject, series of investigations to obtain quantitative and comparable data concerning the influence of various electrolytes upon the activity of pancreatic amylase have been carried out in which the influence of the other factors involved has been recognized and eliminated as completely as possible. In this way it was hoped that the magnitude of the influence, if any, exerted by each salt might be quantitatively established, that other interrelated factors might be recognized, that more light might be thrown upon the way in which such influences are exerted and that

[1] We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

thus more knowledge of the nature and mode of action of the enzyme itself might be obtained.

The study should also be of value in establishing and making it possible to maintain the most favorable conditions for future measurements of enzymic activity.

The results of one series of experiments in which several typical "buffer" salts were studied have already been published.[2] These results led to the conclusion that the influence upon the activity of pancreatic amylase which had hitherto been attributed to such salts as the phosphates, citrates and borates of sodium is mainly if not entirely due to the influence which they exert upon the hydrogen-ion activities of the systems. This explanation would, however, not apply to all electrolytes and the study has, therefore, been extended to include several representative neutral salts.

In the many previous investigations on the influence of neutral salts on the activity of pancreatic amylase, it has generally been assumed that the optimal hydrogen-ion activity for the enzyme as determined in the presence of one concentration of a salt is also the optimal hydrogen-ion activity for that enzyme in the presence of any other concentration of the salt under consideration. Moreover, when there has been any attempt to compare the influence of different salts on the activity of pancreatic amylase there has been no attempt to determine the optimal concentration for each of the salts studied.

Recent investigations[3] have indicated a need for investigating these assumptions as it has been shown that the factors which influence enzymic activity are much more dependent upon one another than has heretofore been generally recognized.

Experimental

The experimental work may be divided into three parts. (1) A study of each salt was made first to determine the influence of changes in its concentration on the optimal hydrogen-ion activity for the hydrolysis of starch by pancreatic amylase. (2) The optimal concentration of each salt was then ascertained by comparing the hydrolysis of starch by pancreatic amylase in the presence of different concentrations of the salt, the enzymic activity being measured at the optimal hydrogen-ion activity in each case. (3) It was then possible to make direct comparisons of the hydrolysis of starch by pancreatic amylase in the presence of each of these salts with the starch in each case adjusted to the hydrogen-ion activity and to the concentration of salt which is most favorable for pancreatic

[2] Sherman, Caldwell and Dale, THIS JOURNAL, **49**, 2596 (1927).

[3] (a) Hahn and co-workers, *Z. Biol.*, **71**, 286, 302 (1920); (b) **73**, 10 (1921); (c) **74**, 217 (1922); (d) **76**, 227 (1922); (e) Myrback, *Z. physiol. Chem.*, **149**, 1 (1926); (f) Sherman, Caldwell and Adams, THIS JOURNAL, **49**, 2000 (1927).

amylase activity. The results of the first phase of this work will be reported briefly in this paper while the other phases will be treated more fully later.

This first series of experiments, therefore, was undertaken to determine the interrelation of hydrogen-ion activity and concentration of salt in the activation of pancreatic amylase. This was accomplished by making parallel measurements of the enzymic activity in the presence of systematically graded hydrogen-ion activities for each concentration of each salt studied.

The salts studied were sodium, potassium and lithium chlorides, and the bromide, fluoride, nitrate, chlorate, sulfocyanate and sulfate of sodium. These were carefully purified by recrystallization.

Because of the difficulties involved in obtaining sodium iodide free from traces of iodine, this salt was omitted from the present series. In our opinion it is impossible at present to separate the influence upon enzymic activity which may be attributable to the iodide ion from that of the traces of free iodine also present in solutions of sodium iodide.

Three preparations of pancreatic amylase were used for these investigations: a commercial pancreatin of high amylase activity obtained by the courtesy of Parke, Davis and Company in 1922, and two purified preparations, one prepared according to the method of Sherman and Schlesinger,[4] the other according to the same method except that the addition of maltose before dialysis was omitted and the final alcohol solution was dialyzed for twelve to fourteen hours instead of forty hours. The activities of these preparations expressed on the Sherman and Kendall[5] scale were 280, 940 and 1130, respectively.

The method of measuring the amylase activity was by the gravimetric determination of the reducing sugar produced from the starch by the enzyme, as shown by the reduction of Fehling's solution and the formation of cuprous oxide. The details of this method have been described in previous publications from this Laboratory.[6] The concentration of each enzyme preparation was kept constant throughout the series of experiments, the solutions being made up immediately before use in ice-cold water containing 0.0005 *M* disodium phosphate.

Selection of a Suitable Buffer.—In order to make a quantitative study of the influence of these neutral salts it was desirable to have present some buffer salt which would not itself influence the enzymic activity but by means of which it would be possible to control accurately the hydrogen-ion activities of the substrates. Mixtures of the phosphates of sodium had been found, as shown above,[2] to answer these requirements. This finding, that phosphate has no influence upon the activity of pancreatic amylase in half-hour hydrolyses of 2% starch at 40°, has been confirmed and extended.

[4] Sherman and Schlesinger, THIS JOURNAL, **33**, 1196 (1911); **34**, 1104 (1912).

[5] Sherman, Kendall and Clark, *ibid.*, **32**, 1093 (1910).

[6] Sherman and Walker, *ibid.*, **41**, 1866 (1919); Adams, *Dissertation*, Columbia University, **1927**.

In the absence of any neutral salt but in the presence of a total phosphate concentration of 0.01 M, the enzyme solutions in the concentrations in which they were used throughout this investigation produced no measurable hydrolysis of starch. An unfavorable hydrogen-ion activity[3e] of the system was not a factor here as the measurements of enzymic activity were made in the presence of systematically graded differences in hydrogen-ion activities from P_H 5.7 to P_H 7.7.

When the concentration of the commercial pancreatin was doubled, there was evidence of slight enzymic activity. This was greatest in solutions corresponding in hydrogen-ion activity to P_H 5.3 but was very slight at best and was evidently due to the small amount of electrolyte impurities present in the commercial pancreatin, because, under similar conditions, the purified enzyme preparations produced no measurable hydrolysis of starch. These results justified the use of 0.01 *M* phosphate buffer mixtures throughout this investigation.

The total phosphate concentration was held constant in all the starch solutions but the relative amounts of primary and secondary phosphates were varied in order to obtain the desired hydrogen-ion activities. The hydrogen-ion activities were measured electrometrically for each starch solution used.

Results with Individual Salts.—The results with sodium chloride are shown graphically in Fig. 1. It is seen from these results that the optimal hydrogen-ion activity for pancreatic amylase activity in the presence of sodium chloride is different for each concentration of sodium chloride as this is increased from 0.0005 to 0.01 *M*. Above 0.01 *M*, the optimal P_H is the same (P_H 7.1 – 7.2) with increasing concentrations of sodium chloride.

TABLE I

A SUMMARY OF RESULTS WITH DIFFERENT SALTS SHOWING THE INTERRELATIONSHIP BETWEEN CONCENTRATION OF SALT AND HYDROGEN-ION ACTIVITY (EXPRESSED AS P_H) IN THEIR INFLUENCE UPON THE ACTIVITY OF PANCREATIC AMYLASE[a]

Concn. of salt, *M*	Most favorable hydrogen-ion activity for pancreatic amylase in the presence of different concentrations of each of the following salts, P_H						
	NaCl	KCl	NaBr	$NaNO_3$	$NaClO_3$	NaSCN	NaF
0.0005	6.5	.....	...	.....	.....	.....	.....
.001	6.7	.....	...	.....	.....	.....	.....
.0025	6.9	.....	...	.....	.....	.....	.....
.005	7.0	7.0–7.1	...	6.6–6.8	6.5	.....	.....
.01	7.1	7.1–7.2	7.1	6.9–7.1	.....	.....	.....
.02	7.1	.....	...	.....	.....	.....	.....
.03	7.1	7.1–7.2	...	.....	.....	.....	.....
.05	7.1	7.1–7.2	7.1	7.0–7.2	6.9–7.1	6.5	.....
.10	7.1	.....	7.1	.....	.....	6.7–6.8	6.3–6.7
.15	...	.....	...	.....	.....	6.7–6.8	.....
.20	...	.....	7.1	7.1–7.2	6.9–7.1	6.7–6.8	6.6–6.8
.30	...	.....	...	.....	.....	.....	6.6–6.8

[a] Mixtures of acid and alkaline sodium phosphates corresponding to a total concentration of 0.01 *M* phosphate were present in all cases.

As shown in Table I, the results obtained with the other salts studied were similar, but the concentration of the salt at which the optimal hydro-

gen-ion activity for the enzymic activity ceases to be appreciably influenced depends upon the salt.

Thus this concentration was found to be 0.01 *M* for sodium chloride, potassium chloride or sodium bromide; 0.05 *M* for sodium nitrate or sodium chlorate; 0.10 *M* for sodium sulfocyanate and 0.20 *M* for sodium fluoride. In each case as the concentration of the salt is decreased below these concentrations, the enzyme is most active in increasingly more acid solutions.

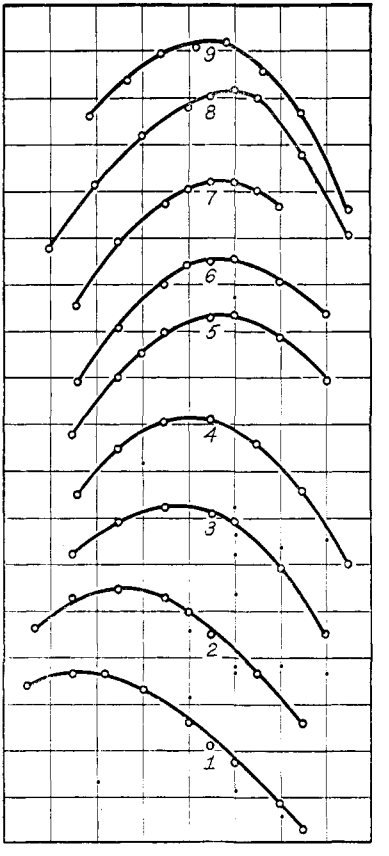
A comparison of the results given in Table I also shows that the optimal hydrogen-ion activity for pancreatic amylase is somewhat different in the presence of these different salts, even when enough of each salt is present to eliminate the influence of changes in its concentration.

Sodium sulfate was found to be without influence upon the activity of pancreatic amylase even when the hydrogen-ion activities of the solutions were varied at graded intervals from P_H 5.7 to P_H 7.7 and the concentrations of the salt from 0.01 to 0.10 *M*.

Because of the insolubility of lithium phosphate it was not possible to study lithium chloride in the same way in which the other salts were investigated and the results with this salt will be given in a later paper.

Conditions for the Enzymic Activity in the Presence of More than One Salt.—Experiments similar to those just described were also carried out with mixtures of two salts. In previous investigations[7] dealing with the influence

[7] Michaelis and Pechstein, *Biochem. Z.*, **59**, 77 (1914); Hahn and Harpuder, *Z. Biol.*, **71**, 286, 302 (1920).

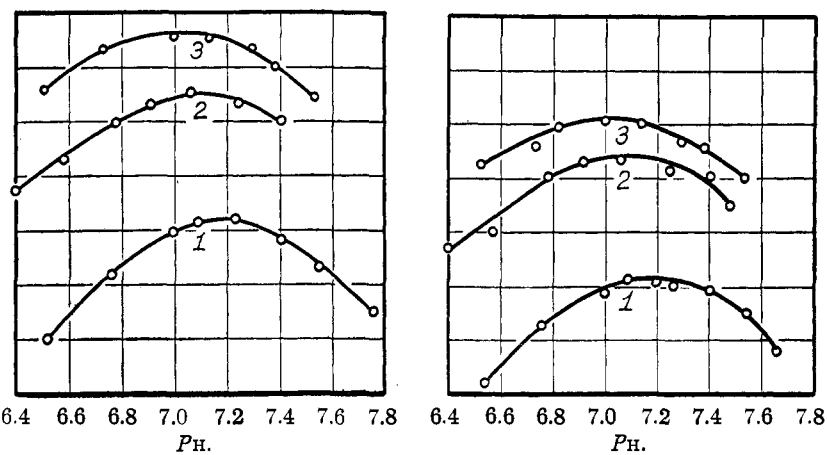


Pancreatin (power 280). 1—0.0005 *M* NaCl; 2—0.001 *M* NaCl; 3—0.0025 *M* NaCl; 4—0.005 *M* NaCl; 5—0.01 *M* NaCl; 6—0.02 *M* NaCl; 7—0.03 *M* NaCl; 8—0.05 *M* NaCl; 9—0.10 *M* NaCl.

Fig. 1.—Influence of the concentration of sodium chloride upon the activity of pancreatic amylase at different hydrogen-ion activities. All the points on any one curve are comparable but the curves are not placed to indicate relative activity of the enzyme in the presence of different concentrations of salt. This is true of the curves in all subsequent figures.

of more than one salt upon the activity of pancreatic amylase no attempt has been made first to establish quantitatively the optimal conditions for the activity of pancreatic amylase in the presence of mixtures of these salts. In the present experiments the activity of the enzyme was measured in the presence of 0.02 *M* sodium chloride with additions of 0.01 to 0.20 *M* sodium nitrate or 0.01 to 0.10 *M* sodium sulfate.

Fig. 2 shows the results obtained with mixtures of sodium chloride and sodium nitrate. The results with mixtures of sodium chloride and sodium sulfate were similar. The optimal hydrogen-ion activity for the enzyme



Pancreatin (power 280).
1—0.01 *M* $NaNO_3$ + 0.02 *M* NaCl;
2—0.10 *M* $NaNO_3$ + 0.02 *M* NaCl;
3—0.20 *M* $NaNO_3$ + 0.02 *M* NaCl.

Purified preparation (power 1130).
1—0.01 *M* $NaNO_3$ + 0.02 *M* NaCl;
2—0.10 *M* $NaNO_3$ + 0.02 *M* NaCl;
3—0.20 *M* $NaNO_3$ + 0.02 *M* NaCl.

Fig. 2.—Influence of the concentration of sodium nitrate upon the activity of pancreatic amylase at different hydrogen-ion activities in the presence of 0.02 *M* sodium chloride.

in the presence of mixtures of 0.02 *M* sodium chloride and sodium sulfate was P_H 7.1–7.2 when the sodium sulfate was present in total concentrations of 0.01 to 0.05 *M*, and P_H 6.9 when the concentration of sulfate present was increased to 0.10 *M*. It was found that as the concentration of sodium nitrate or sodium sulfate was increased the enzymic activity was favored by solutions of greater hydrogen-ion activity, that is, by more acid solutions.

Summary

The optimal hydrogen-ion activity for pancreatic amylase in the presence of different concentrations of sodium or potassium chlorides or of sodium bromide, fluoride, nitrate, chlorate, sulfocyanate or sulfate has been quantitatively investigated.

The optimal hydrogen-ion activity for pancreatic amylase is dependent both upon the kind and concentration of salt present.

In the presence of each of the salts investigated here, the optimal hydrogen-ion activity for pancreatic amylase decreases with increasing concentration of the salt up to a certain salt concentration, beyond which it ceases to be appreciably influenced.

The concentration of salt at which the optimal hydrogen-ion activity for the enzymic activity ceases to be appreciably influenced depends on the salt.

In the presence of more than one of these salts the optimal hydrogen-ion activity depends upon the concentration of the salts present.

Sodium sulfate and sodium phosphate were found to be without influence on the activity of pancreatic amylase.

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THE INFLUENCE OF CONCENTRATION OF NEUTRAL SALT ON THE ACTIVATION OF PANCREATIC AMYLASE[1]

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In a previous paper[2] the optimal hydrogen-ion activity for pancreatic amylase in the presence of different concentrations of each of a series of neutral salts has been reported. These having been established, it was possible to continue the investigation and to determine the optimal concentration of each salt for the activity of the enzyme. The results of this phase of the work will be reported briefly in this paper.

Experimental

A series of parallel hydrolyses of starch by pancreatic amylase was carried out in the presence of different concentrations of each salt with the hydrogen-ion activity in each case adjusted to that previously found to be optimal[2] for the enzyme in the presence of the concentration of salt being studied. The conditions for making up solutions and measuring enzymic activity as there described[2] were maintained throughout. Fig. 1 shows the results obtained from a direct comparison of the activity of pancreatic amylase in the presence of from 0.005 to 0.10 *M* sodium chloride. The enzyme was found to exert its optimal activity in the presence of from 0.02 to 0.05 *M* sodium chloride.

With potassium chloride the concentrations investigated were 0.01,

[1] We are greatly indebted to the Carnegie Institution of Washington for grants in aid of this investigation.

[2] Sherman, Caldwell and Adams, THIS JOURNAL, **50**, 2529 (1928).