connection is not the absolute weight of hydrochloric acid or pepsin present but the relation of one to the other, and the resultant hydrogen-ion concentration.

Summary.

It has been shown in previous papers from this laboratory, as well as by other investigators, that trypsin may be incubated with HCl of P_H = 1.5 through half an hour or longer without appreciable loss of strength. In presence of pepsin the tryptic power is rapidly lost.

However, if sufficient protein is likewise present the acid, in combining with it, is unable to destroy in the same degree. When the acid concentration is reduced in this manner to P_H = 2.6, or below, tryptic activity persists, even through several hours at the temperature of the body. This is a practical condition which very commonly obtains in the human stomach. An active tryptic ferment would unquestionably pass with the chyme, in part at least, into the duodenum where the P_H value is quickly reduced to 6.5, or lower, and there be able to produce a normal proteolytic digestion of some degree.

From the above experiments it appears further likely that some actual protein splitting is accomplished by trypsin at a P_H concentration of 1.8 with certain types of proteins. The rapidity of this proteolysis must be slight, however, and the practical importance low.

Our thanks are due to Mr. H. V. Atkinson for assistance in part of this investigation.

CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF COLUMBIA UNIVERSITY, NO. 268.]

EXPERIMENTS UPON THE AMYLASE OF ASPERGILLUS ORYZAE.

BY H. C. SHERMAN AND A. P. TANBERG.

Received June 19, 1916.

The amylase produced by the fungus *Aspergillus oryzae* has been known to science since about 1875 after having been used empirically in Japan for centuries. It forms the chief active constituent of taka-diastase, introduced by Takamine in 1898,[1] which is prepared by growing the fungus on wheat bran, extracting with water and precipitating by the addition of alcohol in such quantity as to give a concentration of 70% alcohol by volume.

The present study was undertaken with the object of purifying the amylase as far as practicable and comparing its nature and properties

[1] *J. Soc. Chem. Ind.*, **17**, 118.

with those of the pancreatic and malt amylases previously studied in this laboratory.[1]

The taka-diastase used as the starting point in our work consisted of three lots kindly furnished us by Parke, Davis & Company, and a smaller lot, of considerably higher diastatic power than the other three, for which we are indebted to Dr. Jokichi Takamine. These four lots are designated in the following pages as Samples I, II, III, and IV, and possessed diastatic powers ("new scale") of 18, 23, 18 and 118, respectively. The material was a fine, white or yellowish white powder. The first three samples were soluble to the extent of about eighty-five per cent., while Sample IV was almost completely soluble, in ten parts of cold water.

The starch used in all determinations of saccharogenic and amyloclastic power was Merck's soluble potato starch prepared by the Lintner method.[2] The moisture which the air-dry starch contained was determined and allowed for. In determining activities of enzyme preparations allowance was also made for the slight reducing action of the starch upon Fehling solution. The very slight acidity of the starch was corrected by adding to each starch solution as prepared enough hundredth-normal alkali to make it exactly neutral to rosolic acid indicator.

In the use of triple-distilled water, special glassware, etc., and in the technique of determination of diastatic powers (both amyloclastic and saccharogenic) the methods and precautions described in connection with previous work in this laboratory[3] were followed.

Effects of Added Electrolyte.

In order to establish the conditions for determining the activity of this amylase a number of experiments upon the effects of added electrolytes were performed, since the statements on this point to be found in the literature appear contradictory and are in most cases only qualitative. In the present investigation the effects of the various electrolytes tested were studied by careful quantitative determinations of saccharogenic or amyloclastic power in the absence of the electrolyte and in the presence of accurately measured additions in the same manner as in the experiments upon malt amylase previously reported.[4] For the sake of economy of space, however, the data of the individual measurements will not be reproduced here but only the general results obtained with each of the added electrolytes will be given.

Upon the Saccharogenic Action, *sodium chloride* and *potassium chloride* had very little, if any, effect. The activity seemed to be very slightly

[1] THIS JOURNAL, 32, 1073, 1087; 33, 1195; 34, 1104; 35, 1617, 1784, 1790; 37, 623, 643, 1305.

[2] Lintner, *J. prakt. Chem.*, [2] 34, 378.

[3] Sherman and Thomas, THIS JOURNAL, 37, 627, 628, 634.

[4] Sherman and Thomas, *Ibid*, 37, 628–641.

accelerated by these salts at a concentration of 0.0005 molar but the difference was scarcely greater than the probable error of experiment. At concentrations of 0.01 molar and above there appeared a very slight retarding effect. *Primary sodium* (or *potassium*) *phosphate* had no appreciable effect upon the saccharogenic activity of commercial taka-diastase, but when tested upon some of the purified amylase preparations described below, primary sodium phosphate in concentrations of 0.002 to 0.014 molar was found to have a slight accelerating action, the optimum being at 0.005 to 0.008 molar. *Hydrochloric* and *sulfuric acids* (tested upon commercial taka-diastase) appeared to have a scarcely perceptible accelerating action at very low concentrations, not above 0.0001 normal. At higher concentrations of these acids the action of the enzyme was retarded. *Phosphoric acid* exerted a similar scarcely perceptible activating effect at 0.0003 molar concentration and a retarding influence when added in larger quantity. *Secondary sodium phosphate* had a well marked inhibitory effect.

Upon the Amyloclastic Action the effects of added electrolytes were found to be qualitatively similar to the effects on saccharogenic activity, but quantitatively they were much more pronounced. On account of the relatively large experimental error, neither the optimum concentration of the electrolyte nor its quantitative influence upon the activity could be measured with any great exactness. *Sodium and potassium chlorides* at optimum concentrations of 0.02 to 0.08 molar augmented the amyloclastic action to about ten times that observed in the absence of added electrolyte. *Primary sodium and potassium phosphates* at concentrations of 0.02 to 0.026 molar also increased amyloclastic action about ten fold over that found without added electrolyte. *Hydrochloric and sulfuric acids* at concentrations of 0.0001 to 0.0002 normal increased amyloclastic action about eight fold. *Secondary sodium phosphate* had a decided inhibitory effect upon the amyloclastic action of the enzyme.

Purification Experiments.

After the experiments described above had sufficiently established the conditions influencing the activity of this amylase, experiments were undertaken upon the purification of the enzyme, guided by quantitative measurements of the activity of the products obtained.

The material used in most of the experiments was commercial taka-diastase, but for a few of the latest experiments we used material of higher activity furnished by Dr. Takamine.

The method developed in this laboratory for the purification of pancreatic amylase[1] was first applied and yielded a product (Preparation 1) having a diastatic power of 150 ("new scale"). The method which had

[1] Sherman and Schlesinger, THIS JOURNAL, 34, 1105 (1912).

been found successful with malt amylase[1] was then tried. This product (Preparation 2) had a power of 90 ("new scale").

After these preliminary experiments, the purification was studied step by step, as follows:

I. Fractional Precipitation with Alcohol.—A study of the fractions obtained by the addition of increasing amounts of alcohol showed that the fraction of greatest activity was that which precipitated between 60% and 65% of alcohol (by volume). By a single such fractional precipitation it was possible to obtain a Preparation, 5*a*, with a diastatic power of 118. Dialysis with subsequent precipitation increased the power of this fraction to 128 (Preparation 8*a*).

II. Fractional Precipitation with Alcohol and Sodium Chloride.—In the course of the work on precipitation with alcohol it was found that often when the addition of alcohol alone was ineffective, a precipitate could be obtained on the addition of a little sodium chloride. In most of the later preparations sodium chloride was, therefore, used for this purpose, and was found, not only to increase the yield, but also to result in a more effective fractionation. Thus, in Preparation 14, the 60–65% alcohol fraction had a power of 179, and dialysis preceding the precipitation with alcohol and sodium chloride increased the power still further to 193.

It was also found in Preparation 12, that the sticky precipitates often obtained could be readily "hardened" by treatment with absolute alcohol and ether. This treatment not only facilitated the drying, but gave a material lighter in color and of slightly higher power. This procedure was followed, therefore, in all subsequent preparations.

In Preparations 17 and 19 the final precipitate was further fractioned into two parts, that precipitated at 60–62.5% and that at 62.5–65% of alcohol; but this additional fractionation did not result in any improvement of the final product.

Acetone was used instead of alcohol in Preparation 18, but no advantage was found in this modification of the method.

III. Precipitation with Ammonium Sulfate.—If the active material of taka-diastase is at all similar in chemical nature to the amylases of pancreatin and malt, the method of purification should include precipitation by some precipitant which would separate protein from carbohydrates. In Preparation 21 the procedure consisted in precipitation with ammonium sulfate, dialysis of a solution of the precipitate thus obtained and reprecipitation with alcohol. By this means there was obtained a preparation with a power close to 500, *i. e.*, about thirty times the diastatic power of the original material.

The fact that the highest power was obtained in the 60–65% alcohol

[1] Sherman and Schlesinger, THIS JOURNAL, **35**, 1621 (1913).

fraction before precipitation with ammonium sulfate, and after such treatment in the 65–70% fraction, seems to indicate either that the other substances originally present exert a considerable influence upon the solubility of the enzyme in mixtures of alcohol and water, or that the nature of the active material is altered when it is precipitated and redissolved.

All of the preparations except those numbered 20, 22 and 23, were made from the three lots of commercial taka-diastase described above. In making these three (20, 22 and 23), Sample IV was used. This material gave by simple precipitation with alcohol and sodium chloride, a 60–65% alcohol fraction with a power of 392, while the procedure described above, involving precipitation with ammonium sulfate, yielded three precipitates with powers over 500.

Data of Typical Experiments.

Preparation 15.—20 grams of Sample II dissolved in 200 cc. water; freed from insoluble residue by centrifuge; alcohol added to 60%, precipitate rejected, alcohol added to 65% and resulting precipitate dissolved in 65 cc. water; dialyzed in collodion sacs against 1000 cc. water for 37 hours at 5° to 7°, the dialyzate being changed at the end of 14 hours. The dialyzed solution was precipitated with 3 volumes of 95% alcohol, centrifuged and the precipitate washed with absolute alcohol and ether and dried in desiccator over sulfuric acid. Yield, 0.45 gram; Power 193 ("new scale").

Preparation 20.—10 grams Sample IV dissolved in 100 cc. water and purification continued as in Preparation 15 except that time of dialysis was 23 hours. Yield, 0.275 gram; Power 392 ("new scale").

Preparation 21.—10 g. Sample III, precipitated from solution in 100 cc. of water by 60 grams of ammonium sulfate. Precipitate dissolved in 80 cc. water, dialyzed against 800 cc. distilled water for 37 hours, with changes at end of 11 and 18 hours. Precipitated with alcohol and sodium chloride:

(*a*) 0–60% (774 mg. sodium chloride)..... Power, *52*
(*b*) 60–65% (325 mg. sodium chloride)..... Power, *191*.
(*c*) 65–70% (132 mg. sodium chloride), yield 0.1 g. Power, *498*.

Preparation 22.—20 g. Sample IV, 200 cc. water, precipitated by 120 g. ammonium sulfate. Precipitate dissolved and dialyzed for 40 hours. Reprecipitated with alcohol as follows:

(*a*) 0–60%..... Power, *209*.
(*b*) 60–65%, yield 0.5 gram..... Power, *502*.
(*c*) 65–70%, yield 0.2 gram..... Power, *545*.

Preparation 23.—10 g. Sample IV, precipitated with ammonium sulfate, redissolved and reprecipitated with ammonium sulfate, dialyzed against distilled water, and precipitated as follows with alcohol and sodium chloride:

(*a*) 0–60%..... Power, *108*.
(*b*) 60–65%..... Power, *236*.
(*c*) 65–70%, yield 0.1 gram..... Power, *535*.

Composition and Reactions.

The following table gives a brief summary of certain analytical data obtained on the three samples of commercial taka-diastase used, and on four laboratory preparations of increasing activity:

Designation.	Diastatic power. "New scale."	Moisture. %.	Ash (dry basis). %.	Nitrogen (calculated to dry, ash-free material). %.
Sample I.....	18	3.69	12.18	1.63
Sample II.....	23	3.33	14.95	2.69
Sample III.....	18	5.72	18.49	2.38
Preparation 10.....	81	7.10	5.39	3.81
Preparation 15.....	193	5.98	2.42	5.37
Preparation 20*b*.....	392	4.11	2.50	8.13
Preparation 22*b*.....	502	5.29	2.13	10.84

It will be noted that there is a very slight decrease in ash content as the power of the preparations increases from about 200 to over 500. The amount of ash in these preparations is comparable with that observed in malt and pancreatic amylase preparations obtained by somewhat similar methods of purification.

The increase in nitrogen content with increase in power shows that the substances which the purification process rejects contain less nitrogen than the enzyme-rich material which is retained, and suggests that the enzyme is composed either wholly or in part of nitrogenous material.

Especial interest attaches, therefore, to the behavior of the purified preparation when subjected to color reactions which are more or less characteristic of proteins as a group. The Hopkins-Cole test for tryptophan, the Millon reaction and the xanthoproteic reaction were applied to a typical purified preparation (No. 22) and all gave pronounced positive results.

This preparation also gave a blue-violet biuret test. When a solution of the material was boiled a coagulum appeared which gave a blue-violet biuret, while the filtrate from this coagulum gave a pink biuret test.

Relation between the Saccharogenic and Amyloclastic Powers.

In analogy with the comparison previously given of pancreatic and malt amylases,[1] the following data obtained on typical preparations made from taka-diastase are of interest:

Designation.	Power. "New scale."	Wohlgemuth figure.	"Amyloclastic power."	"Saccharogenic power."	Ratio.
Sample I.....	18	30,000	300	42	7 : 1
Preparation 15.....	194	266,600	2,660	438	6 : 1
Preparation 20*b*.....	392	715,000	7,150	880	8 : 1
Preparation 21*c*.....	498	910,000	9,100	1104	8 : 1
Preparation 22*c*.....	545	1,125,000	11,250	1200	9 : 1

"Amyloclastic power" shows the parts by weight of starch completely hydrolyzed to products giving no blue or violet color with iodine after digestion at 40° for thirty minutes.

"Saccharogenic power" shows the parts of maltose formed, at 40° in thirty minutes, by the enzyme acting on an excess of starch under

[1] Sherman and Schlesinger, THIS JOURNAL, 35, 1784 (1913).

the conditions described in the method for determining the saccharogenic power.

The figures show that the amylase of *Aspergillus oryzae* resembles pancreatic amylase qualitatively in that purification does not materially change the ratio of amyloclastic to saccharogenic power. Quantitatively, the ratio is much larger than that observed in the case of pancreatic amylase, in the case of which the relation remains approximately constant at about 2 : 1.

Stability.

No systematic study was made of the stability of solutions of taka-diastase or of amylase preparations obtained from it, but the following facts have been noted during the course of the investigation:

1. A solution of Sample I stood exposed for several days during the summer to a temperature of about 32° without any decrease in activity.

2. A solution of Preparation 12*d*, having a power of 104 at the time it was made up, had a power of 97 after standing in a refrigerator for seventeen days.

Summary.

The Amylase of *Aspergillus oryzae* exerts its maximum activity, both amyloclastic and saccharogenic, in a very slightly acid medium. Acid phosphate accelerates, while alkaline phosphate retards, the action.

Addition of neutral electrolytes, such as sodium and potassium chloride, to commercial taka-diastase, has no measurable effect upon the saccharogenic power, but does increase the amyloclastic action.

The best preparations were obtained by extracting with water, precipitating with ammonium sulfate, dialyzing, and finally precipitating fractionally with alcohol. Such preparations have about thirty times the activity of the commercial material from which they are prepared, but are not so active as purified pancreatic amylase. They have higher amyloclastic, but lower saccharogenic, power than the most active malt amylase preparations yet recorded.

The high ratio of amyloclastic to saccharogenic power which is characteristic of commercial taka-diastase is shown in at least equal degree by the most highly purified preparations.

The purified material resembled the preparations of pancreatic and malt amylases in most of its chemical properties. It gave typical protein reactions when submitted to the Millon, xanthoproteic, tryptophan and biuret tests. Heated in water solution it underwent coagulation, and the coagulum and filtrate both showed the biuret reaction, the color being considerably pinker in the case of the filtrate than with either the coagulum or the original material. That its nitrogen content was lower than that of the best preparations of pancreatic and malt amylases may be due either to a difference in the chemical nature of the enzyme itself,

or to the presence of other substances which the methods of purification thus far developed do not wholly remove.

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CORRECTION.

On page 1231, of the June number, in the first column of Table I, read "Conc. KCl" instead of "Conc. HCl."

NEW BOOK.

Qualitative Analysis. Vol. I of Analytical Chemistry, Based on the Eighth Edition in German by F. P. TREADWELL, PH.D. (Polytechnic Institute of Zürich). Translated and Revised by WILLIAM T. HALL, S.B. (Massachusetts Inst. of Technology). 4th Ed. xiii + 538 p. New York: John Wiley & Sons, Inc. Cloth, $3.00.

The fourth edition of this well-known text on qualitative analysis merits favorable reception as a thorough and convenient laboratory guide. Treadwell-Hall always has been a favorite text, both for the student and the practical chemist, combining, as it does, compactness with sufficient detail. It is not too unwieldy for the student who wishes to go somewhat below the surface in this subject and still covers the ground sufficiently well to meet all the usual requirements of a reference text.

The scope and volume of this edition is, in general, the same as that of previous editions. The introductory or theoretical part covers 75 pages, 117 pages are devoted to the reactions of the cations, 138 to those of the anions, 32 to systematic analysis and 64 to the reactions of the rarer elements.

In revising the book for the 4th ed. Professor Hall has rewritten, and materially added to, the theoretical portion in particular. As stated in the preface, the text is not a literal translation of the German "Treadwell" although it is kept along the same general lines, and in sympathy with the views of Professor Treadwell. Other well-known texts, as Noyes, Stieglitz, Böttger and Ostwald have been drawn upon and indebtedness to these authors is acknowledged.

The theoretical portion is furnished with valuable tables (solubility products, oxidation potentials) and examples in connection with the discussion, which is clear and to the point. The present reviewer hails with pleasure the complete adoption, in this text, of the valence method for balancing oxidation and reduction equations. While there is room for discussion, in general, of the question whether oxidation can *actually* be represented by simple electronic transfer in every case, it is obvious in many cases, and probable in the majority, that this is the actual mechanism. At all events, whoever has had occasion to fight the bug-bear of oxidation and reduction reactions on the part of students, employing long-