is always more stable than pancreatic amylase when simply standing in pure water solution.

On the other hand, pancreatic amylase is the more stable in 50% alcohol solution, and shows a more sustained activity in long-digestion experiments, in which there is present a relatively large amount of substrate together with such a concentration of "activating" electrolytes as had been found best adapted to each enzyme in the ordinary determination of diastatic power.

Both amylases deteriorate during dialysis, and much more rapidly at room temperature than at 5° to 10°. The increased deterioration in dialysis over simple standing in water at the same concentration is more readily demonstrable in the case of malt amylase.

The problem of the chemical nature of the enzymes is discussed in the light of the new osbervations on composition, coagulation, dialysis, activity, and deterioration.

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STUDIES ON THE ACTION OF EREPSIN.

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Since the discovery of erepsin by Cohnheim,[1] in 1901, it has been investigated principally from the point of view of the physiologist. The properties of erepsin and the nature of its action as an enzyme, independent of any function it may have in the animal organism, have been less studied.

Erepsin is known to readily attack peptones and polypeptids, resolving them into their constituent amino acids. Only a few proteins have been found to be digested to any great extent, casein being the most notable example.

Maximum ereptic activity is manifested in weakly alkaline media. Vernon,[2] in studying the action of erepsin on Witte peptone, found that, while small quantities of alkali accelerated the digestion by erepsin, in higher concentrations it injured the enzyme to some extent. He concluded, that "over a certain range of alkalinity these two opposite influences nearly neutralize each other."

For ereptic experimentation, pure intestinal juice, or, a solution of

[1] *Z. physiol. Chem.*, **33**, 451–65 (1901); **35**, 134-140 (1902); **49**, 64–71 (1906); **51**, 415–24 (1907).

[2] *J. physiol.*, 30, 330–70 (1903).

the enzyme prepared by extracting the macerated mucous coat of the small intestine with water or weakly alkaline solution, has been used. The latter preparations have been found to be more active.[1]

No satisfactory method for standardizing the ereptic power of residues has ever been proposed. The activity of ereptic preparations has usually been only estimated by approximately determining the extent of digestion by them of polypeptids or peptones.

Experimental.

1. Determination of Amino Nitrogen in Peptone Solutions.—Witte peptone was found to be easily hydrolyzed by erepsin, and was used in all following experiments as substrate.

The hydrolysis of substances of peptone character is most accurately followed by determining the quantity of free amino nitrogen produced in the solution in the splitting up of the peptone molecules. Two methods for this determination have been principally used: the titration method proposed by Sörensen,[2] and the gasometric method of Van Slyke.[3]

The results obtained from these two methods have been compared by some investigators. White and Thomas[4] preferred the Van Slyke method for their work. Abderhalden and Kramm,[5] also Rogozinski,[6] believed that the Sörensen method gave more reliable results.

Van Slyke pointed out that. among other things, the temperature of the reagents had an influence upon the apparent amount of nitrogen liberated in his apparatus. It seemed necessary to study the effect of temperature on the reaction in the apparatus, as well as the effect of time, which was shown by Abderhalden and Kramm to be considerable. The influence of the rate of shaking the apparatus was also determined.

The titration by the Sörensen method was carried out as suggested by the author. To 20 cc. of the peptone solution were added the neutralized formaldehyde-alcohol mixture and thymol phthalein; this was titrated with 0.2 *N* NaOH solution.

The Van Slyke apparatus was set up as described by the designer. It was connected with a motor, and during the reaction was shaken at the rate of about 250 vibrations per minute; the lower part of the mixing chamber passed through a distance of about three-fourths of an inch. Ten cc. portions of peptone were run each time, and 0.5 cc. amyl alcohol was added to prevent frothing.

[1] Hamburger and Hekma, *J. physiol. path. gén.*, **4**, 805–19 (1902); Salaskin, *Z. physiol. Chem.*, **35**, 419–25 (1902).

[2] *Biochem. Z.*, **7**, 45–101 (1908).

[3] *J. Biol. Chem.*, **9**, 185–204 (1911); **12**, 275–84 (1912).

[4] *Ibid.*, **13**, 111–16 (1912–13).

[5] *Z. physiol. Chem.*, **77**, 425–34 (1912).

[6] *Ibid.*, **79**, 398–414 (1912).

Experiment 1.—Witte peptone solution contained 3 g. per 120 cc. Result of the titration by the Sörensen method—4.28 mg. amino nitrogen per 10 cc.

Results obtained in the Van Slyke apparatus:

Temp. of reagents,	22.5	22.5	24	24	28	28°	
Time of reaction,	6	8	6	8	6	8	minutes
Amino nitrogen per 10 cc.,	4.03	4.40	4.12	4.51	4.34	4.72	mg.

Experiment 2.—In this experiment a peptone solution was used that had been digested with erepsin for some time. Titration by the Sörensen method gave 19.26 mg. amino nitrogen per 10 cc.

Results obtained by the Van Slyke method:

Temp. of room and reagents,	15	15	15	23	23	27	27°	
Time of reaction,	7	9	11	5	7	5	7	minutes
Amino nitrogen per 10 cc.,	19.12	19.77	19.77	19.84	20.52	20.28	21.27	mg.

Experiment 3.—To determine the effect of the rate of shaking the apparatus upon the quantity of gas evolved. The same peptone solution was used as in Experiment 2.

Rate per minute,	190	250	300	
Time,	7	7	7	minutes
Temperature,	23	23	23	
Amino nitrogen per 10 cc.,	14.41	20.52	21.20	mg.

From these experiments it must be concluded that the Van Slyke method for the determination of amino nitrogen gives unreliable results unless extreme precaution is taken to keep all the conditions constant. The accuracy of results obtained by the formol-titration method of Sörensen is in no way affected by the method of manipulation; furthermore, it has been found that a number of determinations of amino nitrogen may be made more rapidly by the titration method than by the use of the Van Slyke apparatus. For these reasons, the Sörensen method for the determination of amino nitrogen has been used in the following experiments.

2. Preparation of the Erepsin-containing Material.—For the most part the procedure suggested by Cohnheim was followed. The mucous coat of the small intestine was ground in a mortar with sand, 0.1% sodium carbonate solution, and a little toluol, and finally allowed to stand at 38° for from one to ten days. This digest was made acid with acetic acid and filtered, whereby a clear, yellowish solution was obtained. To this was added saturated ammonium sulfate in the proportion of three parts to two of the enzyme solution. The residue, thus obtained, was removed by filtration and preserved in a desiccator over sulfuric acid.

In preparing a solution from this material, small quantities were shaken with 0.1% sodium carbonate solution, and dialyzed against flowing water for three to five days. (This length of time has always been found sufficient for the removal of ammonium salts.) This solution was used in digestion experiments with peptone. An aliquot portion was evaporated to dryness, and the loss on ignition of the solids determined. This weight has been taken as the quantity of enzyme present in the solution.

3. Action of Erepsin on Solutions Containing Varying Amounts of Peptone.—Peptone solutions of different concentrations were prepared, and allowed to be acted on by equal quantities of erepsin. In this, as well as in all succeeding erepsin digestion experiments, liberal use was made of toluol as preservative, and the digestion temperature was always 38°

AMINO NITROGEN PER TEN CC.

At beginning.	3.08	4.07	5.14 mg.
End of ten hours.	5.47	6.61	7.81 mg.
Increase.	2.39	2.54	2.67 mg.

It is seen that the amount of amino nitrogen split off from the peptone molecule by erepsin varies considerably with the initial concentration of the peptone solution. Therefore, in all following experiments care was taken that the digesting solutions were of the same concentration with reference to peptone. This was brought about in the following way: A peptone solution was prepared by boiling four or five grams of Witte peptone per 100 cc. of water, cooling, and filtering. Amino nitrogen was determined in the filtrate. For digestion experiments this peptone solution was diluted so that it finally contained 3.5 mg. amino nitrogen per 10 cc. A determination of total nitrogen by the Kjeldahl method shows such a solution to contain 33.46 mg. nitrogen per 10 cc.

4. The Digestion of Peptone by Erepsin as Affected by the Degree of Alkalinity.—In the following experiments the quantity of peptone in solution, also the quantity of erepsin, remained constant; the quantity of sodium hydroxide was varied. Digestions were run for ten hours.

Experiment 1.—

CC. of 0.2 *N* NaOH per 60 cc.,	1	2	4	5	6
Increase in mg. amino nitrogen per 10 cc.,	2.34	2.66	2.66	2.17	1.44

Experiment 2.—

Cc. of 0.2 *N* NaOH per 60 cc.,	1.7	2.0	2.5	3.0	3.5
Increase in mg. amino nitrogen per 10 cc.,	1.85	1.85	1.83	1.85	1.80

There seems to be no definite point which would be considered optimum for ereptic action. The addition of 1.7 to 4 cc. of 0.2 *N* NaOH per 60 cc. of the digesting solution accelerates the digestive action to about the same extent; and, the activity is seen to be diminished where alkali is added in greater or less quantities.

In all succeeding experiments, digesting solutions contain 2 cc. 0.2 *N* NaOH per 60 cc.

5. Action of Alkali on Peptone.—Vernon found that sodium carbonate had a slight hydrolytic action on peptone as measured by the biuret method. In the following experiment a solution of peptone was prepared of the same strength used in the erepsin determinations; each 60 cc. contained 2 cc. of 0.2 *N* NaOH. The solution was preserved with toluol, and allowed to stand at 38°. At intervals, portions of the solution were

titrated by the formol-titration method and the amount of aminonitrogen calculated.

Time,	0	1	4	10	34 days.
Amino nitrogen per 10 cc.,	3.50	3.53	3.53	3.53	3.50 mg.

There is no hydrolysis of peptone by alkali of the strength used in erepsin digestions.

6. Course of the Digestive Action of Erepsin on Peptone with Regard to Time.—In this experiment the amounts of peptone and sodium hydroxide in the solutions are constant, and the amount of erepsin is varied: A, 3.81 mg.; B, 7.62 mg.; C, 13.33 mg. per 60 cc. of solution. Results are expressed in mg. amino nitrogen per 60 cc.

Time, 0	4	7	10	13	24	52	144	840 hrs.
A, 21.18	25.68	28.62	32.76	33.84	42.66	54.26	89.46	123.50
B, 21.18	28.92	34.02	40.80	43.50	56.34	75.26	113.40	133.56
C, 21.84	34.80	42.18	50.82	55.68	72.43	93.32	120.96	135.24

By plotting the quantity of amino nitrogen in solution with time (see Fig. 1), it is seen that the "curves" are nearly straight until after ten

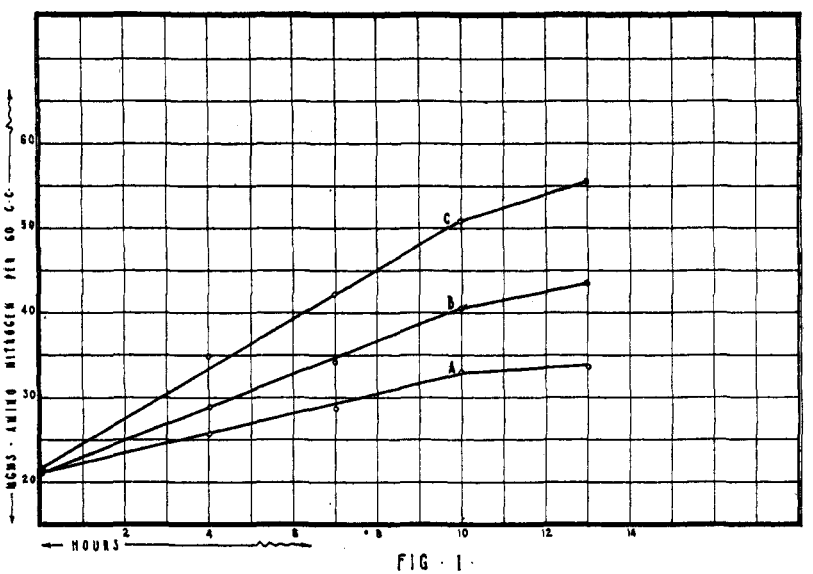

FIG. 1.

hours, *i. e.*, the quantity of amino nitrogen split off by the enzyme is nearly proportional to the time, at the beginning of the reaction. After ten hours, however, there is less and less hydrolysis per unit of time and the curves approach one another (Fig. 2). This latter phenomenon was also pointed out by Bayliss in the action of trypsin on caseinogen.[1]

Since hydrolysis seemed to proceed at a uniform rate during the first

[1] Bayliss, "The Nature of Enzyme Action."

ten hours, digestions were run for this length of time in the following experiments where comparative results were required.

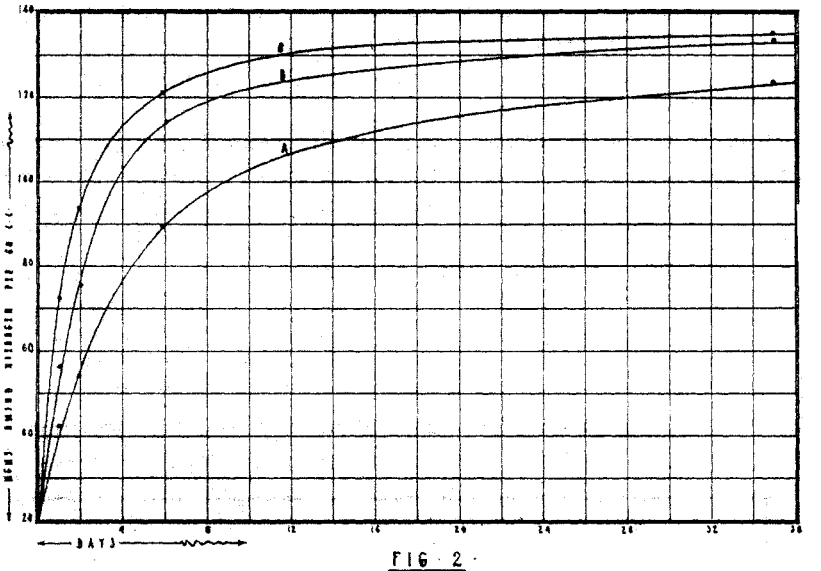

FIG 2

7. Digestion with Varying Amounts of Erepsin.—All solutions were prepared as follows: Enough strong peptone solution was measured out to contain 21 mg. of amino nitrogen. To this was added 2 cc. 0.2 *N* NaOH, the erepsin solution, enough water to make 60 cc., and about 8 drops of toluol. Digestions were allowed to proceed for ten hours at 38°. At the beginning and end of the ten hour period, 20 cc. portions were titrated, and the quantity of amino nitrogen calculated in mg. per 60 cc.

Mg. erepsin per 60 cc..	1.25	2.50	5.00	7.50	10.00	15.00	20.00
Mg. amino nitrogen produced,	6.00	10.08	16.80	22.68	28.20	37.02	44.68

In the study of the action of erepsin on deuteroalbumose, Kutscher and Seeman[1] found the amount digested to be proportional to the square root of the concentration of the erepsin. This may be represented by the formula

$$N/E^{1/2} = \text{constant},$$

where N is the amount of hydrolysis produced by E, the amount of erepsin.

If, however, the amount of hydrolysis is proportional to the amount of active enzyme, as has been found in the case of some enzymes, the following formula should hold:

$$N/E = \text{constant}$$

In the following table the above figures have been applied to these

[1] *Z. physiol. Chem.*, **35**, 432–58 (1902).

formulae: (N and E are taken in mg. amino nitrogen and erepsin per 60 cc., respectively).

E,	1.25	2.50	5.00	7.50	10.00	15.00	20.00
N,	6.00	10.08	16.80	22.68	28.20	37.02	44.88
$N/E \times 100$:	480	403	336	302	282	247	224
$N/E^{1/2} \times 100$:	537	637	752	828	891	955	1003

This would indicate that neither the direct proportion nor the square root law hold; but the ratio which would most nearly yield a constant should be found between the two.

The ratio, which has been found to apply for nearly all erepsin preparations studied, is $-N/E^{3/4}$ = constant. In the following table the above results are applied to this formula:

E,	1.25	2.50	5.00	7.50	10.00	15.00	20.00
N,	6.00	10.08	16.80	22.68	28.20	37.02	44.88
$N/E^{3/4} \times 100$:	508	507	502	500	501	485	474

This rule is seen to hold quite well within the limits of experimental error, where the extent of hydrolysis is not too great. In the last two solutions the reaction proceeded so rapidly that the accumulating products have checked the action of the enzyme.

In a similar manner the results obtained on page 323 may be used:

E,	3.81	7.62	13.33
N,	11.58	19.62	28.98
$N/E^{3/4} \times 100$:	425	428	416

Still another preparation gave similar results:

E,	5	10	15	20
N,	9.12	15.96	21.12	26.58
$N/E^{3/4} \times 100$:	273	284	277	281

In all the above cases the erepsin was obtained from the dog. The following results were obtained on erepsin extracted from the horse intestine:

E,	9.8	14.7	19.6	29.4
N,	6.90	9.60	11.82	17.10
$N/E^{3/4} \times 100$:	125	128	127	135

In the following table numbers I and II show the rule to apply to the action of erepsin obtained from the rabbit, erepsin from the cat (III) shows similar results. The figures under IV were obtained for a solution of erepsin from a dog, which had stood at about 12° for one year.

	I.		II.		III. Cat.		IV. Dog.	
E.....	9.8	9.96	11.8	23.6	4.5	9.0	4.2	8.4
N.....	19.6	16.68	9.42	15.48	4.13	6.47	7.14	12.24
$N/E^{3/4} \times 100$....	180.0	179.0	148.0	145.0	133.0	125.0	243.0	248.0

It is not claimed that erepsin will act according to this law on all substrates and under all conditions, but under the peculiar conditions of these

experiments the law has been found to hold well within the limits of experimental error.

8. A Method for Comparing Ereptic Power of Extracts.—As has already been pointed out, if a solution of Witte peptone of such strength that there is present 21 mg. amino nitrogen per 60 cc., containing also 2 cc. 0.2 *N* NaOH, be acted upon by erepsin for ten hours, the amino nitrogen liberated will be proportional to the weight of erepsin raised to the three-fourths power, provided, further, that there is not greater enzyme activity than which would liberate more than 30 mg. of amino nitrogen per 60 cc. In other words, $N/E^{3/4}$ is constant under the conditions stated, where N = the amount of amino nitrogen produced, and E = weight of erepsin extract.

In the following experiments where activity of erepsin preparations was to be compared, the constant—$N/E^{3/4} \times 100$—was determined for each; quantities N and E were taken in milligrams. This value was designated as *ereptic power*.

9. Purification of Enzyme Extracts.—Very few attempts have been made to prepare erepsin in any state of purity. Cohnheim tried several methods of purification but was unable to get a preparation free from protein. Euler[1] and Raubitschek[2] used special methods for preparing erepsin solutions from the mucous coat, but have not compared the power of these preparations with those obtained by the simpler methods first suggested by Cohnheim.

Cohnheim used an alkaline physiological salt solution for extracting the enzyme from the mucous coat. Erepsin-containing residues were precipitated from these solutions by the addition of saturated ammonium sulfate in the proportion of three parts to two of the enzyme solutions. Some experiments were made to determine the effect of carrying out these details upon the ereptic power of the preparations finally obtained.

Experiment 1.—Equal portions of the mucous coat were digested with equal quantities of the following: A, 0.1% sodium carbonate solution; B, water; C, 0.1% acetic acid solution. Erepsin extracts were obtained from these with ereptic power as follows:—A 144, B 144, C 88.

The use of 0.1% sodium carbonate solution containing 0.9% sodium chloride, and of a solution of 0.1% sodium carbonate alone, was tested similarly. The former solution yielded an extract with ereptic power, 215, and the latter, 248.

It seems that there is no advantage in digesting the mucous coat with alkaline solution, nor in using an alkaline solution containing sodium chloride, over digesting with pure water. Digestion with weak acid seems to injure the enzyme slightly.

Experiment 2.—In this experiment three extracts were prepared: A.—After the digestion of the mucous coat with 0.1% sodium carbonate solution, the mixture was

[1] *Z. physiol. Chem.*, **51**, 213–25 (1907).

[2] *Z. exp. Path. Ther.*, **4**, 674–80 (1907).

acidified and filtered, and a portion was dialyzed for ten days. (This length of time was found necessary for the removal of substances titratable by the formol-titration method.) B.—To a portion of the filtrate obtained in A, ammonium sulfate was added to one-third saturation. This was filtered, the residue discarded, and to the filtrate ammonium sulfate added to two-thirds saturation. Upon again filtering the residue was suspended in 0.1% sodium carbonate, dialyzed, and filtered. C.—To a portion of the filtrate obtained in A, ammonium sulfate was added to two-thirds saturation at once, and the residue treated as in B. The ereptic power of the enzyme in solution A was found to be 153, solution B 180, solution C 180.

These results indicate that there is no advantage in fractional precipitation with ammonium sulfate, and that only slightly more active residues may be obtained by the use of ammonium sulfate at all. In another experiment the erepsin in the original solution after dialysing was even more active than that obtained by precipitating with ammonium sulfate.

Other conditions being identical, it has been found that the longer the time of contact of the macerated mucous coat with the solvent, the more powerful the erepsin preparations obtained. Mucous coat from a cat was divided into two portions and each digested with 0.1% sodium carbonate solution: (1) for five days, (2) for ten days. The ereptic power of the preparation finally obtained from (1) was found to be 175, and that from (2), 240. It has usually been found that very little is gained by extracting the mucous coat more than ten days.

Sörensen[1] obtained erepsin solutions by pressing the juice from the mucous coat by means of the Büchner press.

In two cases, pressed juice was obtained from portions of the mucous coat by means of the Büchner method.[2] These solutions were precipitated by saturated ammonium sulfate solution. Residues thus obtained exhibited much less ereptic power than those obtained by the solution method. Furthermore, by extracting the cake remaining behind in the press, with 0.1% sodium carbonate, it was found that the greatest part of the erepsin had been adsorbed, and that this portion was more active than that which was pressed out.

Other methods that were tried for preparing more active erepsins, *i. e.*, preparations with less admixture of impurities, included the Jacoby uranyl phosphate method,[3] precipitation of the enzyme or its impurities along with cholesterol,[4] precipitation with alcohol, and extracting the erepsin-containing precipitates with organic solvents. In none of these instances was there obtained a preparation of greater ereptic activity than that resulting from the direct solution of the mucous coat and dialyzing.

[1] *Biochem. Z.*, **7**, 45–101 (1908).

[2] Abderhalden, *Handbuch der chemischen Arbeitsmethoden*, **3**, 394.

[3] *Z. physiol. Chem.*, **30**, 135–48 (1900).

[4] Schrumpf, *Beitr. chem. Physiol. u. Path.*, **6**, 396–97 (1905).

Adsorbents were found useful by Michaelis[1] in separating invertin from its protein admixture. The following experiment exhibits the behavior of erepsin in contact with kaolin and hydrated aluminum oxide:

To each of three equal quantities of erepsin solution was added 1 g. of kaolin. One (A) was made slightly acid with hydrochloric acid; a second (B) was made alkaline with sodium hydroxide; the third (C) remained neutral. All were made up to the same volume, shaken five minutes, and filtered. Ereptic activity of the filtrates was determined with the following results:

E	(A), 5.7	(B) 17.3	(C) 10.2
N,	0	14.03	10.07
Ereptic power,	0	165	176

The original solution had an ereptic power of 160. Kaolin in neutral or alkaline solution seems to remove more impurity than enzyme, however, the increase in ereptic activity produced by this treatment is not of such magnitude that it may be considered very important in preparing more powerful residues.

A suspension of hydrated aluminum oxide was prepared by adding ammonium hydroxide to a solution of aluminum acetate, and washing the precipitate by decantation several times. In a manner similar to that described above, the action of this on erepsin in acid, alkaline, and neutral solution was tested. It was found in all three cases that the enzyme was completely adsorbed from its solution by the hydrated aluminum oxide.

The results obtained in this experiment are of interest principally in the consideration of erepsin as a colloid.

10. Action of Erepsin on Blood Fibrin.—Certain investigators have found blood fibrin to be slightly digested by erepsin, others have concluded that there was no action. Cohnheim believed that fibrin was not attacked. Kutscher and Seeman found slight action. Vernon believed that erepsin had little or no action. Weinland[2] found that fibrin was slightly digested by extracts made from the intestinal mucous coats of the pig, however, he believed this to be due to the presence of trypsin which he was unable to wash from the intestines with which he worked.

In the following experiment the action of erepsin on dried blood fibrin was tested. Portions were added to erepsin solutions, some sodium hydroxide solution added, and the whole made up to 50 cc. In all cases toluol was added as preservative. Digestion was allowed to proceed at 38°. At the end of the digestion periods, the solutions were filtered, brought to the neutral point using neutral red as indicator, and the amino nitrogen in solution determined by the titration method of Sörensen.

[1] *Biochem. Z.*, **7**, 488–92 (1908).

[2] *Z. Biol.*, **45**, 292–97 (1903).

Experiment 1. Effect of varying the degree alkalinity.—In each case there was present 1 g. of fibrin, and 33 mg. of erepsin per 50 cc. To A was added 0.25 cc. 0.2 *N* NaOH, to B 1.5 cc. In the blank (C) was used 1.5 cc. 0.2 *N* NaOH, but no enzyme. The digestion ran three days. Upon determining the quantities of amino nitrogen in the filtrates, the following results were obtained: A 2.8 mg. per 10 cc., B 0.42 mg., C 0.28 mg.

It is seen that in a very weakly alkaline solution, erepsin attacked commercial fibrin quite vigorously, however, where 1.5 cc. 0.2 *N* NaOH was added, the quantity of amino nitrogen split off was very little greater than that in the blank. It has been found that the action of trypsin upon fibrin is very little hindered by the presence of NaOH in the higher concentration. Equal quantities of trypsin acting upon one gram of fibrin for three days produced 4.62 mg. of amino nitrogen per 10 cc. of filtrate when 0.25 cc. 0.2 *N* NaOH was present in 50 cc., and 4.20 mg. when 1.5 cc. was present. These facts can be made use of in testing erepsin preparations for presence of trypsin. In the following case this is illustrated:

A solution containing 23 mg. of erepsin (from horse), and 1.5 cc. 0.2 *N* NaOH was made up to 50 cc., and 1 g. fibrin added (A). Another solution (B) was prepared similarly, 5 mg. trypsin being added instead of erepsin. A third preparation (C) contained 23 mg. of the same erepsin, 5 mg. trypsin, and the same quantity of sodium hydroxide. The blank (D) contained only sodium hydroxide and fibrin. Digestion was run three days. Filtrate from A was found to contain 0.63 mg. amino nitrogen per 10 cc.; B, 1.74, C, 2.17 mg.; D, 0.56 mg.

Experiment 2. Action of Erepsin on Fresh Blood Fibrin.—A quantity of fibrin was obtained by whipping fresh horse blood. This was washed, first in flowing water to remove the main part of the blood, then, ground in a mortar to break up the large clumps, and again washed many times by decantation with hot water and 10% sodium chloride solution—finally with hot water to remove all sodium chloride. The action of erepsin and trypsin on this material was tested in a way similar to that described above. In all tests there were approximately equal amounts of fibrin.

(A) contained erepsin and 0.25 cc. 0.2 *N* NaOH. (B) contained an equal quantity of erepsin and 1.5 cc. 0.2 *N* NaOH. In (C) and (D) were equal quantities of trypsin, also 0.25 cc. and 1.5 cc. 0.2 *N* NaOH, respectively. (E) was run as a blank—1.5 cc. 0.2 *N* NaOH, but no enzyme. All solutions were made up to 50 cc. Digestions were run for three days. In the filtrate from (A) was found 1.4 mg. amino nitrogen per 10 cc.; (B), 0.21; (C), 2.17; (D), 2.31; (E), 0.

This shows that erepsin has considerable action of fresh blood fibrin, also; and it is seen again that the higher concentration of alkali almost completely checks the action of erepsin but does not hinder the digestive action of trypsin on fibrin.

11. Action of Erepsin on Gelatin.—Hamburger and Hekma,[1] using the intestinal juice of man, found no action on gelatine.

A solution of gelatin was prepared by dissolving five grams in 200 cc. of hot water, and adding 8 cc. of 0.2 *N* NaOH. Digestions were made as follows:

[1] *J. physiol. Path. gén.*, 4, 805–19 (1902).

(1) To 60 cc. of gelatin solution were added 15 cc. (containing 17 mg.) of erepsin; (2) to 60 cc. of gelatin solution were added 15 cc. (containing 15 mg.) of trypsin; (3) was run as blank—60 cc. gelatin solution plus 15 cc. water. Amino nitrogen, as determined by the formol-titration method, was per 10 cc.

	(1).	(2).	(3).
At beginning	0.74	1.09	0.67
End of twenty hours	3.29	2.94	0.67
End of two days	4.86	3.18	0.65

The erepsin used in the above experiment was obtained from a rabbit, and it was found by testing its action on fibrin to be free from trypsin. The experiment shows the action on gelatin to be very great, certainly no less than that produced by an equivalent amount of trypsin.

12. **Erepsin as an Autolytic Enzyme.**—Kutscher and Seeman found that if the mucous coat of the small intestine be allowed to stand under toluol or chloroform, autodigestion takes place.

Some observations were made to determine whether or not autodigestion of the mucous coat of the small intestine was in any way related to the presence of erepsin. The ground mucous coat was allowed to stand under water, or weak solutions of acid, or alkali, with toluol as preservative. All solutions were made acid and filtered. Portions of the filtrates were neutralized, using neutral red as indicator, and the amino nitrogen determined by the formol-titration method. The quantity of amino nitrogen was taken as a measure of the extent of autolysis.

In this experiment the intestinal mucous coat of the cat was divided into three portions, and digested for five days at 38° with the following solutions: (1) 0.1% sodium carbonate, (2) water, (3) 0.1% acetic acid. Determination of amino nitrogen in the filtrates gave the following results: (1) 11.2 mg. amino nitrogen per 10 cc., (2) 9.5 mg., (3) 6.8 mg.

If autolysis can be measured by the amount of amino nitrogen produced, these results show that in this case it takes place to the greatest extent in alkaline solution, whereas, in most tissues this process has been found to be most active in acid media.

It has been found that in cases where autolysis of the tissue had been most active, more powerful erepsin preparations could be obtained. The following example illustrates this:

The intestinal mucous coats of two dogs were extracted with approximately equivalent quantities of 0.1% sodium carbonate solution. Filtrate from (A) was found to contain 10.8 mg. amino nitrogen per 10 cc., from (B) 35.9 mg. Ereptic power of the preparation obtained from solution (A) was 200, from solution (B) 440.

These results, together with the fact already mentioned—that somewhat more powerful preparations could be obtained by allowing the initial extraction of the mucous coat to continue for a longer period of time, would lead to the conclusion that autolysis of the mucous coat tissue is related to the activity of the erepsin.

In the following experiment the intestinal mucous coat of a cat was

divided into two portions. From one part, erepsin was prepared in the usual way. This preparation was believed to be free from other enzymes. The fibrin test for trypsin was negative; also, the constant, $N/E^{3/4}$ was found to hold when testing its action on peptone. The remainder of the mucous coat was heated to destroy enzymes present. This was thrown on cheese cloth and carefully washed with water. Only the larger portions of the tissue were then used:

(1) To a part of the mucous coat was added the erepsin, 0.5 cc. 0.2 *N* NaOH and water to make 30 cc.; (2) this was run as blank and contained 0.5 cc. 0.2 *N* NaOH and water only. These preparations were digested ten days at 38°, then filtered. Filtrate from (1) was found to contain 3.4 mg. amino nitrogen per 10 cc., (2) 0.4 mg.

While these results seem to show that erepsin may be the cause of autolysis in the tissue of the mucous coat of the small intestine, it is not proved, however, that, in addition, other autolytic enzymes are not present in the fresh tissue.

13. Permanence of the Enzyme.—The ereptic preparations have generally been preserved in one of two ways: (1) the residue obtained from the precipitation of ammonium sulfate, kept in a desiccator over sulfuric acid; (2) a solution in water that has been dialysed and is neutral, and kept at a temperature of 10° to 15°.

A preparation obtained from the horse, and preserved according to the former method, had an ereptic power at the beginning of 109; at the end of eleven days, 105; thirty-five days, 102; sixty days, 97. Dog erepsin residues gave the following results: ereptic power of the fresh residue, 278; the same, fifty days old, 232. Another preparation from the dog showed an ereptic power of 119 when fresh, but after ten months it had fallen to 58.

Some results on the loss in activity of the dialysed aqueous solution are as follows: A solution containing dog erepsin had, when fresh, ereptic power of 277, at the end of ten days, 262. Fresh solution containing horse erepsin exhibited a power of 100; at the end of ten days, 86.

The following results on two dog erepsin preparations show the change on standing in aqueous solutions for longer periods: Fresh solution, ereptic power, 338 and 425; 8 months old, 248 and 254; 12 months old, 222 and 245.

Vernon recommended dilute alcohol as a preservative for erepsin. However, Cohnheim believed that treatment with alcohol weakened the enzyme. In this experiment a solution of dog erepsin with ereptic power of 320 was divided into two portions and treated as follows: to (1) was added one-half volume of alcohol, and to (2) was added the same quantity of water. At the end of six months the ereptic power of (1) was 196, and of (2) 220. These results show that weak alcohol does have an injurious action on erepsin, and that it is better to preserve the enzyme in water.

14. Effect of Bacterial Growth in Erepsin-containing Solutions.—In a number of cases where the preservative was allowed to disappear from erepsin preparations, or, where the preservative was added in insufficient amounts, very marked bacterial decomposition took place. This was accompanied by the appearance of a tryptic enzyme in the solutions. Accordingly, such solutions became useless for ereptic experimentation; they exhibited strong action on fibrin and the three-fourths law was found to no longer hold.

A portion of a solution which had become contaminated, acting on 1 g. of fibrin for two days, produced 7 mg. soluble amino nitrogen per 10 cc., when there was present 1.5 cc. 0.2 N NaOH. The same solution gave the following results when tested on peptone:

E.	20	30	40	50
N.	11.10	14.10	16.38	18.30
$N/E^{3/4} \times 100$:	117	110	103	97

Some of the ammonium sulfate residues became decomposed, and showed similar results. Likewise, in some cases where the intestine was not used for a number of days after the death of the animal, enzyme preparations obtained, gave results of a similar order. It is therefore necessary to guard against bacterial growth in the preparation of enzyme solution by the liberal use of toluol. Many times thymol may be added to advantage in addition to toluol.

Summary and Conclusions.

Witte peptone is quite readily hydrolyzed by erepsin. The extent of hydrolysis may be measured by determining the quantity of free amino nitrogen split off from the peptone molecules. The Sörensen formol-titration method was found more satisfactory than the Van Slyke method for the determination of free amino nitrogen in peptone solutions.

The quantity of amino nitrogen split off by the action of erepsin on peptone varies with the amount of peptone in solution.

The extent of hydrolysis of peptone produced by moderate amounts of erepsin during the first ten hours is directly proportional to the time.

The quantity of amino nitrogen split off during the first ten hours is proportional to the weight of erepsin raised to the three-fourths power. This was found true for preparations obtained from the dog, cat, rabbit, and horse.

Most active erepsin preparations may be obtained as follows: The small intestine, obtained as soon as possible after the death of the animal, is split open, and washed well with running water. The mucous coat is scraped away with a piece of glass. This is ground with sand and a little water in a mortar, transferred to a 0.1% sodium carbonate solution, and allowed to stand with frequent shaking at 38° for ten days. A preservative must be present. The suspension is then filtered through cheese

cloth, and the filtrate made slightly acid with acetic acid. Upon allowing to stand in a warm place for a short time the suspended matter flocculates, and may be rapidly filtered off, using an ordinary filter. The filtrate is dialysed against flowing water for ten days. A nearly colorless solution is thus obtained, which responds to the biuret test very weakly; it contains only a trace of amino nitrogen. It exhibits ereptic action quite strongly.

Erepsin is completely adsorbed from acid, alkaline, and neutral solutions by aluminum hydroxide; it is also adsorbed by kaolin in acid solution. It is not in the least adsorbed by kaolin from neutral or alkaline solution.

Erepsin attacks fibrin in very weakly alkaline solution. Upon slightly increasing the amount of alkali in solution (1.5 cc. 0.2 *N* NaOH per 50 cc.) there is practically no action. While ereptic action on fibrin is held in check by this strength of alkali, tryptic digestion is scarcely hindered. This fact may be made use of in testing erepsin preparations for the presence of trypsin.

Erepsin hydrolyses gelatin very rapidly.

Experiments indicate that erepsin attacks the tissue of the mucous coat of the small intestine, from which it may be concluded that it is the enzyme, or one of the enzymes, inducing autolysis of that tissue. Autolysis in this case is greatest in alkaline solution.

The residues obtained by precipitation with ammonium sulfate and preserved dry, seem to lose in ereptic power somewhat more rapidly after the first few days than neutral aqueous solutions. Dilute alcohol is a less satisfactory medium for preserving erepsin than water.

Bacterial growth should be prevented in all stages of the preparation of erepsin, since it may be accompanied by the production of proteolytic enzymes.

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ITHACA, N. Y.

ON THE PHYSIOLOGICAL ACTIVITY OF COMBINED HYDROCHLORIC ACID.[1]

BY J. H. LONG.

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The question of the efficiency of hydrochloric acid, when combined with different compounds in the digestion of proteins by pepsin, has

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