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.

The writer wishes to express his thanks to Professor Andrew Hunter at whose suggestion this work was begun, and who took an active interest in its progress. Professor M. Dresbach and Mr. A. E. Livingston of the Cornell Medical College kindly provided most of the animal intestines used.

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

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

received much attention in earlier years but has been settled only in a very general way. The acid combined with inorganic bases is of course inactive in this direction, but as to the behavior of proteins or protein derivatives in combination there was, and there is yet, some dispute.

The early discussions of the question we find between 1880 and 1890, when, by the introduction of new indicators, the conception of acid held by protein could be more easily followed. Slightly earlier than this Hoppe-Seyler had made the observation that hydrochloric acid gradually combines with the products of peptic digestion and that the rapidity of digestion diminishes because of this combination.[1] Danilewski, in 1880,[2] discusses the nature of this acid combination and refers the property to the gradual formation, or liberation, of bodies of amine character. With the progress of digestion the amounts of these would increase. To follow the changes in the amount of free acid or free alkali after treatment of protein with an excess of either, he recommends the use of tropaeolin oo and ooo. His observations were somewhat inexact, however.

The use of phloroglucin and vanillin mixture as an indicator for "free" acid was introduced by Guenzburg in 1887,[3] and the sugar and resorcinol mixture by Boas a little later.[4] Congo red seems to have been recommended for physiological investigations somewhat earlier. Through the aid of these indicators it became possible to study much more conveniently the behavior of hydrochloric acid in artificial digestion processes, as well as in the stomach. The distinction between the behavior of phenolphthalein and methyl orange in the titration of digestion mixtures was gradually cleared up.

It was shown by v. Pfungen[5] and others that the hydrochloric acid which combines with protein is so loosely held that it may be dissociated more or less readily by dilution with water, although there is no separation by dialysis. For this reason the proposed method for the recognition of free hydrochloric acid by the addition of cinchonine and extraction with chloroform must be of no value in the separation of actually free hydrochloric acid in excess over the protein. However, the differences in behavior between the more complex proteins and their derivatives or digestion products was not at this time recognized.

An important addition to the methods for the identification of actually "free" acid was made by F. A. Hoffmann[6] when he proposed to employ the speed of the inversion of cane sugar as a measure of acidity present

[1] "Physiologische Chemie," p. 231.

[2] *Maly's Jahresber.*, **10**, 5 (1880); *Liebig and Kopp's Jahresber.*, **1880**, p. 1033.

[3] *Centralbl. klin. Med.*, **9**, 185 (1887).

[4] *Ibid.*, **9**, 817 (1887).

[5] *Maly's Jahresber.*, **19**, 240 (1889).

[6] *Centralbl. klin. Med.*, **10**, 793; **11**, 521; *Maly's Jahresber.*, **19**, 256 (1889); **20**, 234 (1890).

in such cases where protein is concerned. The method had been used successfully in other mixtures. In these papers the notion of mass action in the combination of acid and protein is brought out sharply, as well as the disturbance through dissociation by water. Substances like peptone hydrochloride and betain hydrochloride dissociate in water and permit the acid to act as physiologically free. But substances like quinine hydrochloride, on the other hand, hold the acid in such combination that it is not available for peptic digestion. The distinction is brought out by E. Salkowski and M. Kumagawa,[1] who were able to show that hydrochloric acid combined with leucine or other amino acid so as to form a chemically neutral salt is still physiologically active, since in aqueous solution the acid leaves the leucine and promotes peptic digestion.

In a later publication, following criticism of this point of view by Rosenheim[2] and Hoffmann,[3] Salkowski[4] goes more fully into the subject and shows the conditions under which the hydrochloric acid may become inactive. This follows when a very large amount of egg albumin or fibrin is digested with a limited amount of the leucine hydrochloride or similar substance. It might have been pointed out that digestion with ordinary free acid would be very slow, or might even fail, under the same conditions. This last view is a contradiction of the one advanced by Schiele,[5] according to which the only active acid is that which is already in combination with protein. The so-called free acid would be useful only in combining with the digestion products formed beyond the albumose stage. As these products of hydrolysis are formed, more and more acid will be taken up. Any excess of acid beyond this may be harmful, or at any rate unnecessary.

The rather numerous investigations which have appeared on the question of the behavior of combined hydrochloric acid leave a number of points still undecided. The real activity of the acid in presence of an excess of protein calls for a fuller study, and especially by aid of the electrometric methods which permit a determination of true acidity without the introduction of anything to disturb existing conditions of equilibrium. In the work to be described below, the digestive activity of hydrochloric acid combined with certain amino acids, on the one hand, and with complex proteins, on the other, is compared. As the experiments in this investigation are practically all comparative, digestion is measured by the simple means of filtration and Kjeldahl determinations of the soluble nitrogen. For the purpose this affords a satisfactory measure of what may be called "digestion."

[1] *Virchow's Archiv.*, **122**, 235 (1890).

[2] *Maly's Jahresber.*, **21**, 221 and 222 (1891).

[3] *Ibid.*, **21**, 204 (1891).

[4] *Virchow's Archiv.*, **127**, 521 (1892).

[5] *Maly's Jahresber.*, **25**, 272 (1895).

1. The Behavior of Betain Hydrochloride.

This substance was chosen to illustrate the behavior of an amino acid-like body, because of its comparatively simple structure, and the ease with which it may be obtained in pure condition. It may be secured from chemical dealers and at the present time is obtainable, also, in nearly pure form under the name of *acidol* from one of the large German chemical companies. The original source is the residue from beet-sugar manufacture. The free hydrate has been given the formula $(CH_3)_3 \equiv N.OH$ $-CH_2COOH$, while the hydrochloride is $Cl(CH_3)_3 \equiv N-CH_2COOH$. For the experiments below the commercial product was crystallized from hot alcohol. The purified salt, on titration with 0.1 N alkali and phenolphthalein, gave a sharp result for the halogen acid present. The end point in the titration with methyl orange is nearly as sharp, but the volume of alkali added is slightly less. In other words, the basic behavior comes slightly in evidence here. The titration with alkali and phenolphthalein is complete. What is the actual [H] ion concentration in solutions of this salt? To determine this a series of solutions were made to contain definite amounts of HCl. These solutions were used in digestion experiments to be given. The hydrochloride used, as crystallized above, had a purity of 98.2%, shown by silver and alkali titrations.

In preliminary experiments on the rapidity of digestion by aid of pepsin I used in one instance 5 g. of fibrin with 4.42% of nitrogen, 50 mg. of pepsin, 500 mg. of the hydrochloride and 50 cc. of water. The mixture was digested at 40°. In two hours the reaction was far advanced, with most of the fibrin in solution, but it was continued fourteen hours longer, sixteen hours in all. The mixture was boiled and filtered, the residue being well washed. In the filtrate the soluble nitrogen was found to be 231.7 mg., as the mean of two experiments. This is greater than the total nitrogen of the fibrin, but must be diminished by the nitrogen of the betain hydrochloride and the pepsin, 45.5 mg. $231.7 - 45.5 = 186.2$. As the total fibrin nitrogen was 221 mg. the result shows a digestion value of 84.2%.

In a similar experiment with eggs I employed the coagulum from 1.2 g. of egg albumin powder containing 70% of dry protein. In the mean of two experiments the nitrogen found was 131.6 mg. This must be diminished by the nitrogen of a blank experiment in which killed pepsin was used. At the end of each digestion the mixtures were exactly neutralized with sodium hydroxide, boiled and filtered. In this way the neutralization precipitate was excluded. $131.6 - 44.1 = 87.5$ mg. of nitrogen from the more advanced digestion, which was practically completed in two hours, but which was continued over night as above.

These experiments indicate an advanced degree of digestion, and

nearly as much as would have been obtained by the use of the equivalent amount of free hydrochloric acid.

For most of the experiments detailed below a solution was used which contained 10.69 g. of the betain hydrochloride dissolved in water to make 500 cc. This corresponds to 2.5 g. of actual HCl in the water volume, or 25 mg. HCl to 5 cc. of solution.

Experiments were now made with a constant volume of liquid, weight of pepsin, weight of egg, but variable acid product, the digestions being carried uniformly through four hours. In each test the weights were as follows:

Coagulum from 1.5 g. egg albumin = 1.06 g. protein.
Pepsin 15 mg. in 30 cc. solution.
Liquid volume made always to 60 cc.

At the end of the digestion, conducted at 40°, the mixtures were boiled and filtered. The slight residues were well washed and the total filtrates used for nitrogen determinations. The volumes of betain hydrochloride solution taken contained the equivalents of 25, 50, 75, 100, 125. and 150 mg. of actual HCl and would furnish, therefore, a considerable amount of nitrogen to the filtrate. The volume in test A was 5 cc., containing 106.9 mg. of the hydrochloride and, therefore, 9.6 mg. of N. This weight is subtracted from the result of the Kjeldahl, and corresponding amounts in the other tests. The pepsin N is too small to be noted here.

The results of the Kjeldahl determinations are given below, and the values are corrected for the betain hydrochloride N. It is assumed that all the nitrogen from this source came through in the filtrate, which is probably correct for a test carried out in this manner. When neutralization is effected before filtering, however, some of the betain may be separated and precipitated with the neutralization product.

TABLE I.

	Cc. betain HCl sol.	Total N.	Betain N.	Net N.	Protein.
A	5	0.0157	0.0096	0.0061	0.0381
B	10	0.0806	0.0192	0.0614	0.3838
C	15	0.1530	0.0288	0.1242	0.7762
D	20	0.1874	0.0384	0.1490	0.9313
E	25	0.1961	0.0480	0.1481	0.9256
F	30	0.2079	0.0575	0.1504	0.9400

TABLE II.

	Mg. HCl added.	Soluble N.	Protein.
A	25	small, not	determined
B	50	0.0980	0.6125
C	75	0.1422	0.8887
D	100	0.1410	0.8812
E	125	0.1526	0.9538
F	150	0.1618	1.0112

A parallel set of experiments was carried out, using free hydrochloric acid in place of the betain hydrochloride of Table I. The conditions of the tests were otherwise the same. It was observed that the digestions began somewhat earlier and for a time appeared to be more marked than with the betain salt. At the end of the period of four hours the extent of the digestion in the two cases was nearly the same, with the advantage slightly in favor of the free acid, as shown in Table II.

As showing, further, the rapid digestive action of the betain hydrochloride experiments were made with fibrin, as with the egg. In each test 3.9 g. of fibrin, equivalent to 1.06 g. of protein, were used. The total liquid volume was 60 cc., containing varying amounts of the betain salt and 15 mg. of pepsin. The digestion time was three hours at 40°. At the end of the time the mixtures were boiled, diluted and filtered for the Kjeldahl determinations. The results of the tests are given in Table III.

TABLE III.

	Cc. betain HCl sol.	Total N.	Betain N.	Net N.	Protein.
A.........	5	0.0462	0.0096	0.0366	0.2287
B.........	10	0.1496	0.0192	0.1304	0.8150
C.........	15	0.1824	0.0288	0.1536	0.9600
D.........	20	0.1869	0.0384	0.1485	0.9281
E.........	25	0.1995	0.0480	0.1515	0.9468
F.........	30	0.2160	0.0575	0.1585	0.9906

The digestions progressed very rapidly and it is evident that the salt dissociates largely and in amount sufficient to produce a change in the protein almost comparable with that of the free acid. That some of this separated acid goes at once into combination with the protein is suggested by the following experiment:

The coagulum from 1.5 g. of egg powder, representing about 1 g. of true protein, was mixed with 30 cc. of the betain HCl solution containing 150 mg. of the acid. The mixture was shaken through two hours to effect complete combination. (Data to be given in another paper will show something about the rapidity of combination of weak acid with protein.) At the end of the shaking, the mixture was filtered and the solid residue washed with about 30 cc. of water. The filtrate, titrated by aid of methyl orange, required 22.6 cc. of 0.1 N NaOH, while 30 cc. of the original required 40.8 cc. Therefore, 40.8 — 22.6 = 18.2 measures the acid held, about 66.4 mg., for the gram of protein. This is essentially the amount which would have been taken up from a weak solution of free hydrochloric acid.

In two similar experiments portions of coagulum from 1.5 g. of egg were mixed with 20 cc. of the betain hydrochloride solution and 50 cc. of water and shaken through one hour, but not as actively as before. The mixtures were filtered, the residues being washed as before. In one filtrate the HCl required 19.6 cc. of 0.1 N silver nitrate equal to 71.5 mg. of the acid, while in the other filtrate 19.5 cc. of 0.1 N NaOH were needed to neutralize the acid. The results agree in showing that 28.5 mg. of HCl had been taken by the protein in this case, where the original concentration of the acid was much stronger than in the first test.

It is desirable to know the actual concentration of the active hydrochloric acid in these solutions used for the digestion experiments, that is, the [H] concentration. Attempts were made to determine this in two ways: First, by the use of a series of indicators as suggested by Friedenthal, Salm and others, and perfected by Soerensen and second, by the measurement of the electric potential in concentration cells. This method seems to give much better results than could be secured by the indicator titrations and was finally followed for all the tests. Cells of the Ham-

burger type were used for a time, but were later given up in favor of the Hasselbalch cell and the 0.1 N calomel electrode, as recommended by Soerensen.[1] The much easier correction for the diffusion potential is a point of great practical importance here, and for this purpose observations were made with 3.5 N and 1.75 N KCl, as a connecting bridge between the concentration cell and the calomel electrode.

For the sake of comparison and to test the general accuracy of the method I made a series of determinations of π_0, the potential of a true normal solution, at 18°, following the Soerensen procedure. This I found to be = 0.3381 at 18°, and about 0.3379 at 20°, which is a trifle higher than the Soerensen value, *viz.*, 0.3377. I kept the temperature as nearly as possible at 20° during the tests, and therefore took

$$P_H = (\pi - 0.3379)/0.0582$$

as the formula for reduction generally employed. For higher temperatures the denominator is a little larger and is practically 0.0002 more for each degree. (The whole denominator is 0.0001987T, where T is the absolute temperature.) As the P_H is the common logarithm of the reciprocal value of the [H] concentrations of the solutions, the latter is easily calculated.

Determinations made on the solutions of the same strength as were employed in the digestion experiments gave these results. The figures given under "theory" show the [H] concentrations on the assumption that the HCl is fully separated from the salt and completely dissociated.

TABLE IV.

	Cc. betain HCl sol.	H_2O.	P_H.	C_H.	Theory C_H.
A	5	55	2.067	0.0085	0.0114
B	10	50	1.868	0.0135	0.0228
C	15	45	1.753	0.0176	0.0343
D	20	40	1.656	0.0220	0.0456
E	25	35	1.592	0.0256	0.0571
F	30	30	1.534	0.0292	0.0686

The experimental results are very interesting. The weakest solution is slightly more than 0.01 N, while the strongest is less than 0.1 N. It must be remembered that the betain hydrochloride is dissolved so as to make in 5 cc. the equivalent of 25 mg. of HCl, and each solution was diluted to 60 cc. with pure water before each test. As the dissociation in the 0.01 N HCl is not far from 95%, it will be seen that in the weakest solution the [H] of the mixture is about 85% of the theoretical. In the strongest solution, the [H] concentration is about 46% of the theoretical.

Several determinations were made of the concentration of the [H] in the acid liquid standing over egg coagulum at the outset of digestion, or before pepsin is added. Two tests are sufficient to illustrate this con-

[1] *Biochem. Z.*, **21**, 131 (1909); *Ergeb. der Phys.*, **12**, 393 (1912).

dition. In the first, 10 cc. of the above betain hydrochloride solution (= 50 mg. HCl) were mixed with 50 cc. water and the coagulum from 1.5 g. of egg. In the second case 30 cc. of the same acid solution were mixed with 30 cc. of water and the 1.5 g. of egg. The mixtures stood two hours with frequent shaking and were then filtered. The potential tests were made on the filtrates, with these results:

	Cc.	P_H.	C_H.
1	10	2.259	0.0055
2	30	1.745	0.0179

In the first case the concentration of the acid left is about what comes from the dissociation of a protein hydrochloride in excess of water. In the second case the hydrogen ion concentration is reduced to that of an original solution of 15 cc. of the betain hydrochloride. The gram of protein has taken up approximately 7% of acid and the supernatant liquid exhibits clearly the excess.

In ordinary digestion experiments in which a protein-like egg albumin is dissolved by pepsin and hydrochloric acid, the concentration as well as the total amount of the latter come into play. We speak of a 0.2% acid as being a good concentration, and this is true, provided the total amount of acid is more than enough to combine with all the protein. In the usual test to measure the activity of pepsin, as given in the U. S. Pharmacopeia, for example, we have a mixture containing about 1.25 g. of real protein with one-tenth its weight of actual HCl in 40 cc. of liquid. The original concentration of the acid is therefore about 0.3%, but approximately one-half of this is taken up by the protein, leaving a residual concentration of 0.15%, which is probably more than is required for maximum activity. From the experiments above with betain hydrochloride, as well as with free HCl, it is seen that digestion is rapid in a far lower concentration.

II. The Behavior of Glutaminic Acid Hydrochloride.

For the experiments here, I used a pure preparation made in the laboratory by the hydrolysis of casein and which, by titration of the chlorine and by a nitrogen determination, was known to be practically pure glutaminic acid hydrochloride, $COOH.CH_2CH_2.CHNH_2.COOH.HCl$. The salt is readily soluble in water and 500 mg. required 54.5 cc. 0.1 *N* NaOH for titration with phenolphthalein. This measures the hydrochloric acid and one carboxyl group.

Preliminary experiments showed that solutions of this hydrochloride in presence of pepsin digested egg and fibrin rather rapidly. In the following four trials the coagulum from 1.2 g. of egg was used in A and B, and 5 g. of prepared fibrin in C and D. In each case 50 mg. of pepsin, 600 mg. of glutaminic acid hydrochloride and 50 cc. of water were used. The mixtures were digested at 40° over night. At the end of two hours the

disintegration was far advanced, and apparently complete over night. Blank experiments were made by using killed pepsin to correspond to each case. The mixtures were boiled, filtered, the filters washed and the filtrates used for nitrogen tests.

TABLE V.

	Total N.	Blank N.	Net N.	Protein.
A	0.1568	0.0358	0.1230	0.7687
B	0.1638	0.0338	0.1300	0.8125
C	0.2471	0.0357	0.2114	1.3212
D	0.2541	0.0357	0.2184	1.3650

The whole of the nitrogen of the pepsin and of the hydrochloride should appear in the blank and should be nearly 50 mg. But it is evident that, with the capture of some of the hydrochloric acid by the protein, the glutaminic acid becomes less soluble and possibly precipitates to some extent. However, the total digestion seems to be from 75 to 80% of what might be theoretically expected, indicating a behavior much like that of the betain hydrochloride.

A number of digestion experiments were then made exactly as with the betain salt. A solution of glutaminic acid hydrochloride was made containing 6.285 g. to 250 cc. Five cc. of this contains 125.69 mg., or 25 mg. of HCl in combination. The N content of 5 cc. is 9.6 mg.

As in the former experiments the coagulum from 1.5 g. of egg albumin was always taken, corresponding to about 1 g. of protein. The digesting volume was always 60 cc., of which 30 cc. were made up of a pepsin solution containing 15 mg. of pepsin. The digestions were carried on through four hours at 40°, and at the end of this time the mixtures were boiled, diluted, and filtered for the nitrogen tests. The actual digestions began somewhat more slowly than with the betain hydrochloride, and evidently do not go as far. This is probably due to the fact that the hydrogen ion concentrations are lower, as will appear below. Table VI gives the result of the determinations for soluble nitrogen.

TABLE VI.

	Cc. glut. HCl sol.	Total N.	Glut. N.	Net N.	Protein.
A	5	0.0196	0.0096	0.0100	0.0625
B	10	0.0711	0.0192	0.0519	0.3244
C	15	0.1211	0.0288	0.0923	0.5769
D	20	lost	0.0384	..	..
E	25	0.1765	0.0480	0.1285	0.8051
F	30	0.1894	0.0575	0.1319	0.8244

It is seen that these digestion values are not as high as were found in the case of the other hydrochloride and the table to be given below will afford a possible explanation of this. Some of the same solution was employed for determination of the hydrogen concentration at 20°, as de-

scribed for the experiments with the betain hydrochloride. Table VII below gives the numerical values.

TABLE VII.

	Cc. glut. ac. HCl sol.	H_2O.	P_H.	C_H.	Theory C_H.
A	5	55	2.216	0.0060	0.0114
B	10	50	2.010	0.0097	0.0228
C	15	45	1.908	0.0123	0.0343
D	20	40	1.852	0.0140	0.0456
E	25	35	1.811	0.0154	0.0571
F	30	30	1.785	0.0164	0.0686

It is plain that we have, in all the dilutions, a lower hydrogen concentration than was the case with the betain salt. In the last mixture, equivalent to 150 mg. of hydrochloric acid in the 60 cc., the concentration is but little over half that of the corresponding betain hydrochloride. This difference undoubtedly accounts for the lower digesting activity observed in the experiment.

The [H] concentrations observed here are practically all due to the dissociation of the inorganic acid, as the dissociation of the glutaminic acid is very low. The residual concentrations of similar solutions, standing over egg albumin, were found to be very trifling in a number of experiments similar to those carried out for free hydrochloric acid to be referred to below.

III. The Behavior of Protein Hydrochloride.

In the foregoing we have seen the behavior of hydrochloric acid combined with small groups comparable to the component structures of the protein molecule itself. The acid unites readily with the complex protein, as it does with the amino acid derivatives, and the question of its physiological action here now comes up. In the therapeutic use of hydrochloric acid it is usually administered in the form of diluted solutions of the free acid, and the only important objection to this is the strongly acid taste. To overcome this objection such bodies as the betain hydrochloride have found favor and it has been shown above that a marked digestive activity is actually present with them. Combinations of proteins and hydrochloric acid have also come into use in recent years, and for these claims of acid strength are made. The experiments to be given below are intended to throw light on this point.

In the investigations I used a form of acid albumin obtained by adding hydrochloric acid to egg albumin and evaporating to dryness at a low temperature. The dry substance was powdered and mixed with enough more albumin powder to bring the HCl content to exactly 5%. The product has a strong acid taste and is only partly soluble in water. The nitrogen content was found to be 12.25%, but when the product is shaken up in water the amount of nitrogen dissolved is not large. Two experi-

ments illustrate this: Two portions of 2 g. were shaken up with 100 cc. of water and incubated at 40° through six hours. To one of these portions 50 mg. of killed pepsin had been added. At the end of the time the liquids were filtered and on the filtrates nitrogen determinations were made.

	Total N.	Sol. N.	Pepsin N.	Net N.
A	0.245	0.0196	..	0.0196
B	0.245	0.0209	0.0008	0.0201

While not a large proportion of the protein is soluble, an appreciable amount of the acid dissociates and may be obtained in the clear filtrate. This is best shown by starting with a larger weight of the powder. 10 g. were shaken up with 250 cc. of water and allowed to settle over night. Part of the supernatant liquid was filtered off and portions of 10 cc. were titrated with 0.1 N NaOH.

10 cc. with methyl orange require 1.1 cc. = 100 mg. HCl for whole.
10 cc. with phenolphthalein require 1.7 cc. = 155 mg. HCl for whole.

This result shows that while some of the acid comes through combined with protein, some must be considered as existing in the form of free acid. This amounts to about 20% of the whole acid in the substance shaken up with water.

In the presence of pepsin the mixture undergoes rather a slow digestion, and the amount of hydrochloric acid now found in the filtrate is increased, but not greatly as the digestion is incomplete. This is shown by Table VIII where the effect of adding more protein is also brought out. In these experiments 2 g. of the powder and 50 mg. of pepsin were added to 100 cc. of water and digested through six hours at 40°. In some of the cases the coagulum from egg powder with 70% of real protein, and fibrin with 29% of real protein, was added. The results were as follows:

TABLE VIII.

	Prot. HCl. G.	Added pepsin. Mg.	Added Protein. G.		Sol. N.	Protein.
A	2	..	...	...	0.0196	0.1226
B	2	50	...	...	0.1472	0.9200
C	2	50	1	egg	0.1117	0.6981
D	2	50	2	egg	0.0790	0.4936
E	2	50	2	fibrin	0.1260	0.7875
F	2	50	3	fibrin	0.1142	0.6937
G	2	50	5	fibrin	0.0825	0.5156

The protein in the 2 g. of the powder used above amounts to about 1.53 g. Slightly over 60% of this digests when no more protein is present, but the addition of either egg or fibrin brings the digested amount down to a much lower figure. With increasing amounts of added protein the digested fraction progressively diminishes. This is undoubtedly because of the binding of the hydrochloric acid. In similar experiments with

other amounts of protein added, no greater weight of protein was digested. It appears, therefore, that acid held in this way is not capable of insuring an active digestion, even of its own protein.

The concentration of the dissociated hydrochloric acid is very low in the mixture of the powder with water. This was observed with the mixture made by adding 10 g. of the powder to water enough to make 250 cc. of solution. The mixture was well shaken through an hour and allowed to stand over night. The acid strength was determined in a cell of the Hamburger type against 0.1 N HCl. The result was found, $C_H = 0.00726$. The insoluble residue was again shaken with water and allowed to stand. The supernatant liquid gave a lower acid value than before. This operation was repeated twice. The results of the four trials were as follows, showing for the H concentrations:

1st water, 0.00726; 2nd water, 0.00517; 3rd water, 0.00483; 4th water, 0.00352.

Assuming the hydrochloric acid to be all split off in the first water treatment the concentration should be 0.0548, or with 95% dissociated 0.05296. The amount of free acid available for activation of pepsin is, therefore, but a fraction of that split off from the betain hydrochloride. A preparation of this character is sold under the name of *oxyntin* as a hydrochloric acid substitute.

This holding of the HCl by protein is shown easily in another way, using moist, freshly coagulated egg albumin as a substratum. I made a series of experiments in which different amounts of coagulated egg were mixed with 150 cc. of $N/15$ HCl and either digested through a certain time or allowed to stand some hours before testing. In all cases the character of the liquid standing over any remaining albumin was determined. The volume of this liquid which could be filtered off was found. The results of experiments are given in Table IX.

TABLE IX.

	A.	B.	C.	D.	E.	F.	G.
Coag. from	5	6	8	10	5	10	15 g. egg
Treatment	Digested	Digested	Digested	Digested	Not digest.	Not digest.	Digested
Vol. filt.	149 cc.	146 cc.	138 cc.	135 cc.	...	...	100 cc.

In A, B, C, D, and G the digestions were carried through 4 hours at 50°, with the addition of 50 mg. of pepsin. In A and B practically all of the protein went into solution before the end of the period. In C some was left which held a little of the digesting liquid and in D the amount was still greater. In G the portion digested was apparently very slight and the volume of liquid which ran through the filter was much less. The weights of actual protein, and acid added, expressed in percentages of the protein weight, are as follows:

TABLE X.

	A.	B.	C.	D.	E.	F.	G.
Weight of protein, g....	3.5	4.2	5.6	7.0	3.5	7.0	10.5
Per cent. HCl..........	10.43	8.69	6.52	5.21	10.43	5.21	3.48

The potential tests in six of the undiluted filtrates, representing the real acid concentration of the supernatant liquids, gave these results:

TABLE XI.

	A.	C.	D.	E.	F.	G.
P_H.................	1.696	2.352	2.636	1.403	1.763	2.957
C_H.................	0.0201	0.0044	0.0023	0.0396	0.0173	0.0011

In A an appreciable amount of free acid is found in the liquid after digestion, while in E, of the same original strength but not digested, the free acid concentration is nearly twice as great. In C there is scarcely enough acid for proper digestion, while in D there was an appreciable amount of albumin left. In G the acid concentration was far too low for digestion, as most of the egg remained undissolved. What passed through the filter was merely acid albumin. In E and F there was, of course, no digestion and very little solution. The amounts of free acid were consequently higher here.

After making the potential tests some of the liquids were returned to the original filtrates and mixed with wash water sufficient to bring all the volumes up to 250 cc. In this way practically everything soluble was washed out of the filters. 25 cc. portions of the filtrates were titrated with 0.1 *N* alkali and with 0.1 *N* silver nitrate, after separation of albumin, with the following results:

TABLE XII.

	NaOH with methyl orange.	NaOH with phenol phthalein.	NaOH formal titration.	$AgNO_3$ titration.	Per cent. of HCl recovered.
A.............	Ca 6 cc.	12.2	14.0	9.7	97
B.............	Ca 3 cc.	13.0	15.4	9.2	92
C.............	Trace	12.8	15.5	8.6	86
D.............	0	13.0	15.4	8.6	86
G.............	0	5.4	6.5	3.5	35

The results with the methyl orange titration were far from sharp, as is always the case in presence of partly digested protein, and merely give an indication of the practical absence of free hydrochloric acid in three of the cases. Figures showing the results of the formaldehyde titration are also added, but they show no marked increase of protein products in the digestion filtrates. No great importance can be attached to them for the further reason that the weak acid concentrations were probably not sufficient to prevent a slight bacterial action between the time of digestion and the titration. But the results of the silver nitrate determinations are interesting. If all the hydrochloric acid had passed

into the filtrates the amount of silver nitrate required would have been exactly 10 cc. in each case. But there was always a loss depending on the amount of acid held by the undigested protein. By *prolonged washing* all of this acid could have been dissociated and washed out. Such acid cannot be considered as mechanically retained, but is doubtless chemically combined. In Sample G two-thirds of the acid is so held, and here the protein residue is very large.

For the actual digestion of protein by pepsin and acid it is not necessary that the amount of free acid should be large, but there *must be some excess*, if the digestion is to be at all rapid. This slight excess may probably come in some cases as a result of dissociation. In Sample C we have about the limit of digestive action, but with the same amount of acid and double the protein there is practically no digestion in G.

These experiments are sufficient to show that combinations of protein and HCl, with not more than 5 or 6% of the latter present, can have no value as digestive agents. This amount of acid is scarcely sufficient to permit the digestion of the protein itself, to say nothing of the digestion of added protein. In this respect the protein-acid combinations are not comparable with the combinations with amino acids. In Experiment A, where the digestion is rather rapid and is practically completed in two hours, the [H] concentration was found to be $P_H = 1.696$. It is interesting to note that this value is between the limits found by Soerensen[1] for the optimum digestion of egg albumin, as measured by a quite distinct process, *viz.*, the determination of the amount of nitrogen in the digestion filtrate which may not be precipitated by stannous chloride. His optimum is given at $P_H = 1.63$. It is evident, however, that any marked increase of albumin in A would lead to a decided decrease in the digestive activity.

In these experiments I have received valuable assistance from Miss Mary Hull to whom my thanks are due.

Resume.

It has been shown in this paper that the hydrochlorides of betain and glutaminic acid dissociate in aqueous solution to sufficient extent to permit the acid to aid pepsin in the rapid digestion of egg albumin or fibrin. This behavior is probably typical of amino acid combinations in general. In the case of the betain salt the action is almost equal to that of dilute hydrochloric acid of the same gross concentration. With glutaminic acid hydrochloride the action is somewhat slower, but still marked. In either case, in the mixture of coagulated protein and the hydrochloride, a part of the HCl will leave the latter and become attached to the protein.

Mixtures made by combining hydrochloric acid with protein, in a sense

[1] *Biochem. Z.*, **21**, 297 (1909).

analogous to the hydrochlorides of amino acids, are physiologically much less active. Such mixtures hold scarcely enough acid to digest themselves perfectly. If further amounts of protein are added, with pepsin, digestion becomes very slow. When the protein and hydrochloric acid are so related as to bring the [H] concentration down to $P_H = 2.96$ the rate of digestion is slow. This is the case when the weight of the acid is about 3.5% of the weight of the egg albumin, and 150 cc. of $N/15$ acid is the liquid volume.

On the other hand, when the weight of the acid in 150 cc. of $N/15$ HCl is about 10% of the weight of the albumin, and the hydrogen concentration of the supernatant liquid is about $P_H = 1.69$, we have very rapid digestion. This appears to be near the maximum of activity. We find all degrees of digestive activity between these limits. Dry preparations of protein and hydrochloric acid about midway between these limits cease to be physiologically active.

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GASTRO-INTESTINAL STUDIES. VII. THE UTILIZATION OF INGESTED PROTEIN AS INFLUENCED BY UNDERMASTICATION (BOLTING) AND OVERMASTICATION (FLETCHERIZING).

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1. Introduction.

Within the last few years attention has been frequently called to the importance of the proper mastication of food. Campbell[1] in 1903 made the following statements:

> "The primary object of mastication is to break up the food so as (1) to facilitate the swallowing of it and (2) to insure its admixture with the digestive juices, both of the mouth and whole digestive canal. Mastication has other far-reaching effects. It promotes the flow of saliva, secures proper insalivation of food, increases the quantity of alkaline saliva passing into the stomach, stimulates the heart and circulation, and, finally, influences the nutrition of the jaws and their appendages by stimulating blood and lymph circulation. Proper mastication tends to diminish the amount of food consumed, by reducing the quantity needful to constitute a sufficiency, for the more perfectly a food is chewed the more perfectly is it digested and the more economically is it disposed of in the system. Insufficient mastication is the cause, direct or indirect, of many evils. It may cause local irritation resulting in acute gastritis; appendicitis is believed by Sir Frederick Treves to be due directly to food bolting, gastric intestinal catarrh may be induced through the action of certain toxins; gastric secretion may be checked through paralysis of stomach nerves, while the teeth and jaw structures are underdeveloped and disposed to disease."

[1] *Lancet*, July 11, 1903.