At first there is no precipitation, but on shaking, a precipitate coagulates out from the solution. If the precipitate be immediately filtered, a dark red filtrate is obtained, which, on standing, becomes hazy and deposits a further quantity of the precipitate, leaving the solution less deep in tint. The precipitate obtained above is filtered, washed with a small quantity of water and dried in the desiccator. The salt consists of a black powder.

Calculated for $2S(CH_3)_3I$, PtI_4: Pt, 17.55; found: Pt, 17.63.

Triethylsulfonium Iodoplatinate.—Unlike the previous salt, this is obtained as a fine chocolate-colored precipitate by adding chloroplatinic acid to triethylsulfonium iodide. The salt is dried as usual in the desiccator and analyzed.

Calculated for $2S(C_2H_5)_3I$, PtI_4: Pt, 16.32; found: Pt, 16.51.

Investigations on similar lines are being continued.

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ON SOME CONDITIONS AFFECTING THE ACTIVITY AND STABILITY OF CERTAIN FERMENTS. II.

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Received June 28, 1913.

In a recent paper[1] we have described the action of weak acids and alkalies on one of the constituents of pancreatin, the amylopsin. In this paper we wish to present facts bearing on the behavior of tryspin, the other important ferment in pancreas preparations, under the same conditions.

Popularly, pancreatin is supposed to contain notable amounts of the two ferments and sometimes the lipolytic enzyme is assumed to be present, but practically only one of these is contained in appreciable amounts in the ordinary commercial products, *viz.*, the starch-converting enzyme. As described in the Pharmacopoeia of the United States, and in other similar works, pancreatin should contain a proteolytic enzyme, active in alkalin or neutral medium, but very few of the manufactured products have any proteolytic power whatever.

From a study of the literature bearing on trypsin and its relations to acids and alkalies it is evident that observers have had in hand extremely weak products only, with a converting power far below that of pepsin of laboratory or commercial manufacture. Attempts to produce trypsin by the processes originally described by Kühne[2] yield at best a very weak product, with which it is difficult to make satisfactory experiments on the behavior of acids and alkalies. The effects are in all cases modified by the presence of relatively large amounts of accompanying inert pro-

[1] THIS JOURNAL, **35**, 895.

[2] *Verhand. des naturhist. med. Vereins zu Heidelberg*, 1877, p. 194. *Untersuchungen aus dem phys. Inst. der Universität Heidelberg*, I, 1878.

teins. Kühne spoke of his products as being able to rapidly digest bits of fibrin, but there is nowhere any evidence as to the relative weights of fibrin and ferment employed. It is likely that he must have used very much more crude ferment than fibrin, unless we accept the somewhat questionable hypothesis of Mays[1] that by accident, almost, Kühne secured extracts of the beef pancreas which were of a potence not since attainable by others. It was not thought necessary at the time of these earlier investigations to add anything to the pancreas powder or extract to bring about activation, and this fact has led to some question as to the real activity of the Kühne products.

Whatever their proteolytic power, however, Kühne mentioned that these ferment substances were extremely sensitive to acids. He stated that in the presence of 0.05% of hydrochloric acid their activity is still held, but above this destruction soon follows. This statement is sometimes misquoted and made to say that in 0.05% acid the ferment is destroyed. It was shown by other workers that a much greater acidity is necessary for actual destruction. Thus, Engesser claimed[2] that the pancreas powder made by him was able to resist an acidity of 0.5% hydrochloric acid. Of the same product Ewald says[3] that in presence of 0.3 % hydrochloric acid it is able to dissolve fibrin to the condition of a turbid solution. These contradictory statements have long been quoted in the literature. It is likely that the last-named writers overlooked the fact that acid of the strength used is sufficient to bring about a partial solution of the fibrin in time. In hydrochloric acid of 0.3% strength fibrin swells to a jelly-like mass, and, if enough liquid is present, slowly dissolves. It is also true that the pancreas powders as made by the Engesser process contained so much protein that they would probably neutralize a considerable amount of acid by combination.

It will be recognized that the question of the action of pepsin on trypsin must depend largely on the behavior of the acid itself with trypsin, since pepsin can be used only in an acid medium. From his experiments Ewald concluded that trypsin is destroyed in acid solutions containing pepsin, and that the rapidity of destruction is proportional to the amount of pepsin present. Engesser admitted that any trypsin present as such would be quickly destroyed by acid and pepsin, but he held that in the pancreas powders the trypsin is present as a "zymogen," which has a greater resisting power toward acid and pepsin.

These views have mainly a historical interest at the present time, but it is proper to note that views almost as divergent on the two main contentions, the concentration of the acid which destroys trypsin, and the effect

[1] *Z. physiol. Chem.*, **33**, 428.

[2] *Z. klin. Med.*, **2**, 192; *Maly's Jahresber.*, 10, 297.

[3] *Ibid.*, 1, 615; *Ibid.*, **10**, 297.

of pepsin and acid on trypsin, are still expressed in recent literature. Part of the difficulty in the problem seems to be due to the character of the various trypsin preparations employed by different investigators. The ferment activity of the pancreas powders made in the laboratory is often very low and the same is true of most of the market products, as already intimated. It is commonly recognized that trypsin products, in general, have but a feeble action on raw egg albumin, but it is often overlooked that the action on boiled egg albumin is also relatively slow, although easily followed with moderately strong preparations. If tested by their action on coagulated egg, many market preparations would have to be pronounced practically inert, while with casein or fibrin a fairly sharp digesting power might be shown, as will be illustrated later. The behavior of acid toward trypsin may be best shown by the use of fibrin or casein as a test material, rather than egg.

In our efforts to determin the action of weak hydrochloric acid on trypsin we have used a number of products. First, a laboratory pancreas powder and a laboratory glycerol extract which had stood long enough to become active; second, samples of Gruebler's trypsin, and finally the products of some American manufacturing laboratories. The best of these, by far, is the "trypsin" of Fairchild Brothers and Foster, New York, which may be recommended for scientific investigations. Specimens which have come under our observation seem to have about four times the digesting power toward fibrin possessed by the Gruebler preparation, which is often taken as a standard for comparison. The "extract of pancreas" of the same firm, F. B and F., is also very active and in the experiments to follow will be often referred to.

The Action of Acid on Trypsin.

As our object here was mainly a practical one, to determin the possible part played by pepsin in aiding the acid destruction of trypsin, we have confined ourselves to a study of the behavior of hydrochloric acid. To learn the effect of pepsin on trypsin it is, of course, necessary to fix first the action of the acid with which the pepsin works and for this purpose hydrochloric acid alone comes practically into consideration. Trials have been made with both egg albumin and fibrin.

The egg albumin used for our experiments was prepared in this way: A very clear, light-colored, dry hen's egg albumin is brought into this country from China, and may be obtained from dealers. It seems to be used mainly by bakers, as are other similar products. Good samples of this product, which are not hard to secure, dissolve in distilled water to an opalescent liquid, free from lumpy particles and free from odor. When this solution is poured into a large volume of boiling distilled water, slightly acidified with acetic acid, a finely divided, perfectly white precipitate is secured, as with the solutions from fresh eggs, with this advan-

tage, however, that the nitrogen content of the washed coagulum is pretty constant. With the very clean albumins which we have been able to secure for this purpose the nitrogen of the washed coagulum amounts to something over 100 milligrams for each gram of the original dry egg. With one lot it was practically 106 milligrams. This weight is more nearly constant than may be obtained by using the coagulum from ordinary storage eggs, and serves well for digestion comparisons. We have found, however, that the results obtained from some other brands of dry egg albumin were not as satisfactory. Much of the American or German "egg" albumin cannot be used at all, as it is not freely soluble.

When fibrin is used, it is prepared by thorough washing in water and disintegration in a meat chopper set to cut very fine. The chopped mass is soaked in glycerol until the larger part of the water is removed, which is hastened by pressing out thoroughly and treating with fresh glycerol. Finally, the mass is pressed as dry as possible and preserved at a low temperature in bottles containing a small amount of toluene. The product may be kept some weeks at a low temperature, and as it is comparatively dry and uniform it may be weighed directly for a series of digestions. For one of the last lots prepared in this way, and used for experiments to be quoted, the nitrogen content of 5 grams was found to be 162.9 milligrams, in the mean, corresponding to about 20% of protein. It has been noticed, however, that if left in a warm room autolytic digestion of the fibrin takes place rather readily, making the product unfit for use.

In the experiments to be given first we employed egg, as described. The sodium carbonate mentioned was always taken as anhydrous. In all cases a correction was made for the nitrogen of the ferment itself.

Experiment A. 50 mg. of F. B. and F. trypsin plus coagulum from 1 gram of egg (106 mg. N) plus 50 mg. of sodium carbonate in volume of 50 cc. Digested 10 hours at 40°. The undissolved egg was filtered off.

Soluble N = 25.5 mg.

Experiment B. 50 mg. of same trypsin plus 30 mg. of HCl made to 50 cc. with water and incubated 1 hour at 40°. Then 100 mg. of sodium carbonate was added with the coagulum from 1 gram of egg and the digestion continued through 10 hours at the same temperature.

Soluble N = 27.2 mg.

Experiment C. 50 mg. of same trypsin, plus coagulum from 1 gram of egg, plus 200 mg. of sodium carbonate made to volume of 50 cc. and digested 10 hours at 40°.

Soluble N = 14.8 mg.

The amount of digestion with the 200 mg. of carbonate is less than when 50 mg. were used in Experiment A.

Experiment D. 50 mg. of same trypsin, plus coagulum from 1 gram of egg, plus 30 mg. of HCl, plus 50 mg. of pepsin, boiled with water, all made to 50 cc. and digested 1 hour at 40°. Added then 100 mg. of sodium carbonate and digested 10 hours at 40°.

Soluble N = 36.4 mg.

Experiment E. Conditions the same as in *D*, but the pepsin solution not boiled. Soluble N = 26.9 mg.

Experiment D seems to show that some digestion must go on in the acid medium before the carbonate is added, the pepsin being here inactive. In Experiment E, however, with the active pepsin present, there is a lessened total digestion, as will be shown more fully in other tests.

Experiment F. 50 mg. of same trypsin, plus 100 mg. of sodium carbonate, plus 3 grams of fibrin (97.7 mg. of N) made to 50 cc., and digested at 40°. The fibrin was all dissolved in 30 minutes.

Experiment G. 50 mg. of Gruebler's trypsin, plus 100 mg. of sodium carbonate, plus 3 grams of the same fibrin, made to 50 cc. volume and digested at 40°. Over 2 hours required for complete solution.

The last experiments show the marked difference between the two kinds of trypsin, which results were duplicated by many other observations, but they show also the ease of fibrin digestion as compared with that of egg. In this respect they are but duplicates of many others we have made. It was found that a still greater degree of acidity in the preliminary incubation seemed to have but a slight action on the trypsin, when tested with fibrin, and this was shown quantitatively with the F. B. and F. ferment in the following tests:

Experiment H. A series of solutions was made containing in 50 cc. 50 mg. of the trypsin and increasing weights of the acid, up to 100 mg. These solutions were incubated 30 minutes at 40° and then mixed with enough sodium carbonate to neutralize the acid and leave 100 mg. of the carbonate present in each case. Enough sodium chloride was added to four of the mixtures to make the content the same as in the mixture with the greatest acid originally. Finally 5 grams of fibrin, containing 162.8 mg. of nitrogen, were added to each mixture and all were incubated 5 hours at 40°. The rate of digestion appeared to be nearly uniform. At the end of the period all of the solutions were filtered and Kjeldahl determinations made on filtrate and residue. The results were as follows:

No.	Mg. of HCl.	Mg. of trypsin.	Soluble N.	Residue N.	Total N.
1.....	60	50	156.6	7.8	164.4
2.....	70	50	152.3	11.5	163.8
3.....	80	50	151.0	11.9	162.9
4.....	90	50	151.4	13.4	164.8
5.....	100	50	151.4	10.6	162.0

It will be seen that a preliminary digestion in a medium containing 0.2% of hydrochloric acid does not destroy or practically retard the activity of the trypsin. The digestion, after neutralization, is as complete in the mixtures which contained the largest amount of acid as it is in the one with the smallest amount. The weights of total nitrogen recovered are not quite uniform. This is doubtless due to the fact that absolute uniformity in the nitrogen content of the moist fibrin could not be secured.

In an experiment similar to number 5 of the table, 25 milligrams of pepsin were added to the 100 milligrams of acid and the 50 milligrams of trypsin. After incubation, neutralization and addition of fibrin, and

further incubation of five hours the residue nitrogen was found to be 77.8 mg. and the soluble nitrogen 85.3 mg., or 163.1 mg. in all, indicating a rather marked effect due to the action of the pepsin on the trypsin.

Experiment I. The preceding experiment was repeated, using a new and much drier preparation of fibrin. 3 grams samples contained, in the mean, 168.8 mg. of nitrogen. In these tests the acid added in the preliminary incubation was greater, as shown in the table.

No.	Mg. of HCl.	Mg. of trypsin.	Soluble N.	Residue N.	Total N.
1.....	100 + 25 pepsin	50	18.2	154.0	172.2
2.....	120	50	129.3	37.5	166.8
3.....	140	50	139.4	25.8	165.2
4.....	160	50	130.7	43.5	174.2

In the first of these tests the preliminary incubation of 30 minutes with pepsin, in addition to the acid present, resulted in a marked lowering of the efficiency of the trypsin in the final fibrin digestion. In the other cases the acid digestion alone, with amounts of acid reaching 0.32%, shows a slight impairment, only, of the activity of the trypsin. For our purpose it was not thought necessary to carry the acidity beyond this last value, or to increase the time of preliminary incubation beyond 30 minutes. We have kept in mind the practical bearing of these questions on problems connected with actual conditions in the human body.

It is evident from the above tests that trypsin, even when not protected by a great excess of protein, is able to withstand an acidity of over 0.3% of hydrochloric acid through 30 minutes at 40°. Somewhat similar results have recently been reported by others, to be referred to, but the weights of protein used in the final tests of the residual trypsin activity have usually been so small as to leave the question really very much in doubt. It will be recognized that in these experiments we are not dealing with the matter of the digestion by means of trypsin in acid solution. It has been shown by many observers that in presence of acid the activity of trypsin is practically nil.[1] We are simply concerned with the effect of acid on trypsin in absence of much protein.

Other Investigations.

Some extended observations have been published by Wroblewski, Bednarski, and Wojczynski[2] on the behavior of ferments toward each other and toward acids and alkalies, in which the conclusions are scarcely warranted by the method of experimentation followed. In one group of experiments recorded, acid was allowed to act on their trypsin preparation through 6 hours at 37°. After neutralization with soda fibrin was added and the digestion of this carried on through 8 hours. From the results of these tests it appeared that acid of 0.14% strength had but little

[1] See, for example, Chittenden and Cummins, *Am. Chem. J.*, 7, 49.

[2] *Beitraege zur chem. Phys. und Path.*, 1, 288 (1902).

action, while a concentration of four times this was found to be very deleterious. The uncertainty in the experiments comes from the failure to state the amount of fibrin used or actually digested. It appears that about 1 cc. of swollen fibrin ("nach Gruetzner") was taken, but in actual weight this is small, while the trypsin weight was apparently relatively large.

In a second series of tests 50 mg. of fibrin powder were employed. This was dissolved in soda and added to the previously incubated trypsin, which had been digested four and one-half hours. With a preliminary acidity of 0.028% but a trace of the fibrin remained undigested, while after incubation with acid of 0.56 and 0.98% most of the fibrin was left, as shown by filtering and weighing the residue. It will be seen that the fibrin weight is very small to begin with and the method of weighing is lacking in accuracy.

In their third series of experiments egg albumin solution was employed, and in each test 3 cc. of a "1 to 4 dilution." No weights are given, but this is apparently equivalent to 90 or 100 mg. of actual albumin. In one test where 50 mg. of "trypsin" were incubated in acid of 0.028% strength, and then the egg added after making alkalin, 54.8 mg. of albumin were recovered, as undigested, by filtering and weighing. After digestion with acid of 0.98% strength the recovered undigested albumin was 69 mg.; but almost the same weight was recovered, 69.8 mg., in a blank where neither trypsin nor acid was employed. The results seem to be without meaning, and it is evident that the authors have overlooked two important facts, first that raw egg is practically not attacked by trypsin,[1] and secondly, that in the digesting alkali, in the strength used by them, raw egg is slightly soluble and probably enough to bring about the slight "digestion" reported.

A longer set of experiments, Table V of their paper, suffers apparently from the same oversight. In these tests it appears that very large weights of trypsin in comparison with the egg are used, and in most cases two to one, or 200 mg. of trypsin to 100, or less, of albumin. Some of the individual tests seem to show that the preliminary incubation with hydrochloric acid weakens the trypsin, as shown by the subsequent action on the egg, while other experiments apparently lead to the opposit conclusion. In one case 200 mg. of trypsin acting on 3 cc. of the egg albumin solution left a residue of 27.2 mg. of unchanged protein, while in a second experiment 60 milligrams of the same trypsin, after incubation with 107.8 mg. of pepsin in 0.028 and 0.56% hydrochloric acid, left residues of 23 and 29.9 mg. of albumin from 3 cc. of the same egg solution. The apparent digestion of the egg may be due to something else than the trypsin.

[1] This fact is brought out by Cohnheim, *Physiologie der Verdauung*, p. 214, and by Hammarsten, *Physiological Chemistry*, 6th ed. (Mandel), 483. We have found it true in our own investigations.

In other experiments in the same series there appear to be digestions of about half the protein, even after the trypsin had been subjected to the action of acid of 1.12% through 6 hours.

We have commented on these experiments at some length, because they have been frequently quoted in the literature, but in view of the discrepancies noted, and others, the conclusions indicated, that trypsin withstands the contact with relatively strong hydrochloric acid, should not be drawn.

A more recent investigation than the one just referred to was carried out by T. Kudo,[1] who employed casein solutions as the substratum for the action of the trypsin. Casein has been frequently used for such experiments because it may be easily secured in practically pure condition. Kudo followed the method of Fuld,[2] according to which a weak sodium caseinate is prepared and treated with the ferment until it no longer gives a precipitate on neutralization with dilute acetic acid. The method is good in principle and easily carried out. In our hands it has given good results. From his experiments Kudo drew the conclusion that extremely weak acids retard the action of trypsin perceptibly, quantities as low as a thousandth of a per cent., even, having an effect easily seen. For actual destruction of the ferment, however, much larger quantities of the acid seemed to be necessary.

In our trials with this method we found it desirable to use a stronger casein solution than that employed by Kudo, making the reaction depend on the digestion of 12 milligrams of casein in place of 4 milligrams. One set of experiments may be quoted as illustrating the whole series of tests.

Experiment J. Our casein solution was made with the minimum amount of alkali required for the formation of the acid salt.[3] As a trypsin we used in this series the "Extract of Pancreas" of Fairchild Brothers and Foster, already referred to. Six test tubes were charged with amounts of the pancreatin, as shown below, and to each was added 5 cc. of 0.2% hydrochloric acid. All the tubes were incubated one-half hour at 40°, then neutralized exactly. To each tube 6 cc. of 0.2% casein solution was added and these, in turn, were incubated one hour and then the tests for unaltered casein were made by adding to each tube three drops of the acetic acid reagent (1 part of acid, 50 parts of alcohol, 49 parts of water). The table below shows the relations. It is seen that even with the weakest pancreatin concentration, and after the half-hour incubation with acid, the casein is completely changed in the final digestion.

No.	Mg. of pancreas.	Mg. of casein.	Result.
1.....	2.0	12	Complete digestion
2.....	1.3	12	Complete digestion
3.....	0.8	12	Complete digestion
4.....	0.5	12	Complete digestion
5.....	0.3	12	Complete digestion
6.....	0.0	12	No app. change

[1] *Biochem. Z.*, **15**, 471 (1909).

[2] *Verh. Vereins inn. Med.*, **1907**; *Maly's Jahresber.*, **37**, 446; **38**, 375.

[3] Long, "Some Investigations on Salts of Casein," THIS JOURNAL, **28**, 372.

These results are in agreement with those obtained by the fibrin method in indicating the limited action of hydrochloric acid of 0.2% strength on the tryptic ferment.

In a second series of experiments 20 milligrams of pancreatin and 5 milligrams of active pepsin (U. S. P.) were incubated in 5 cc. of 0.2% hydrochloric acid 30 minutes. Then the acid was neutralized and portions corresponding to the weights of pancreatin taken above, that is, to 2, 1.3, 0.8, 0.5 and 0.3 mg., were incubated 1 hour with 12 mg. of casein in a total volume of 10 cc. The application of the acid test showed now in all the tubes the presence of undigested casein, and in marked quantity, indicating the complete or partial destruction of the pancreatin by the acid *plus* the pepsin. In this respect our results differ from those of Kudo, which, however, were made with gastric juice and not with known quantities of acid and pepsin of definit strength. This behavior of pepsin is only incidental to the present paper and is merely referred to in passing, because the object of the investigation, originally, was to study this disputed point, which will be handled in detail later.

Action of Water on Trypsin at Different Temperatures.

In pure aqueous solution trypsin becomes inactive much more quickly than pepsin, and markedly so at elevated temperatures. In 50% glycerol trypsin may be kept through months. As to the exact effect of elevated temperatures there is a great lack of uniformity in the views of those who have reported on the subject. From our experience with a considerable number of trypsin preparations we have come to the conclusion that the salts present are probably the most important factors in modifying the stability.

According to the older experiments of Heidenhain,[1] trypsin begins to lose its activity when warmed in aqueous solution to 35°, but this is denied by Kühne and others.[2] Biernacki[3] states that it becomes inactive in slightly alkalin solution at 45°. Much of the discrepancy is doubtless due to the fact that different observers have worked with products of very different degrees of purity, but another fact of equally great moment is this, that the various trypsin preparations described in the literature seem to have been extremely weak products, their activities having been measured by the solution of small amounts of fibrin or coagulated egg in digestions carried through many hours.

In some experiments made to test this behavior in aqueous solution we have obtained the following results, after using the "extract of pancreas" and the "trypsin" of Fairchild Brothers and Foster, and the glycerol extract of our own production.

[1] *Maly's Jahresber.*, 5, 176; *Pflueger's Archiv.*, 10, 557.

[2] "Citation by Oppenheimer," *Die Fermente*, p. 114.

[3] *Maly's Jahresber.*, 21, 248.

Experiment K. In the first table below are given the digestion values obtained by incubating 100 mg. of the pancreatin (extract of pancreas) through 30 minutes at 40°, 50° and 60°, in 50 cc. pure water, after which treatment the liquids were brought to 40° and to each were added 100 mg. of sodium carbonate and 3 grams of fibrin (N content 123.5 mg. in mean). The mixtures were incubated again through 4 hours at 40° Kjeldahl determinations were made on residue and filtrate.

No.	Prelim. temp.	Fibrin. Grams.	Soluble N. Mg.	Residue N. Mg.	Total N. Mg.
1.....	40°	3	103.3	19.3	122.6
2.....	50°	3	77.6	46.5	124.1
3.....	60°	3	19.1	106.0	125.1

It will be seen that the effect of the preliminary digestions at the higher temperatures is to greatly weaken the activity of the pancreatin.

In a similar experiment with a preliminary incubation of 30 minutes at 40°, 25 milligrams of pepsin were added to the neutral solution, along with the 100 milligrams of pancreatin. At the end of the half hour the mixture was acidified by the addition of 100 milligrams of hydrochloric acid to the 50 cc. Three grams of the same fibrin were added and a new incubation was made at 40°. At the end of 45 minutes the digestion seemed to be complete. On filtering and making Kjeldahl determinations we found:

Soluble N.....	117.5 mg.
Residue N.....	4.6 mg.
Total.....	122.1 mg.

Corrections were always made for the nitrogen of the ferments. It is evident that under the conditions the pancreation has not destroyed or appreciably weakened the pepsin activity, as the fibrin digestion is here due to the latter ferment.

Experiment L. In a digestion test similar to K we used the trypsin instead of the pancreatin, 50 milligrams of trypsin being subjected to the preliminary incubation in 50 cc. of water. The fibrin used was not from the same lot as the other. Final digestion, 3 hours.

No.	Prelim. temp.	Fibrin. Grams.	Soluble N. Mg.	Residue N. Mg.	Total N. Mg.
1*a*.....	40°	3	56.0	..	...
1*b*.....	40°	3	57.9	47.0	104.9
2*a*.....	50°	3	39.2	65.9	105.1
2*b*.....	50°	3	38.9	66.1	105.0
3*a*.....	60°	3	14.0	91.0	105.0
3*b*.....	60°	3	14.2	91.0	105.2

It will be noted that in this experiment the time of final digestion is 3 hours in place of 4, and that 50 milligrams of trypsin were used in place of the 100 of pancreatin. But is is evident that the trypsin has suffered relatively more than the other ferment at all temperatures. This seems

to be true of the stronger ferments, in general. As greater activity is secured, it is often at the expense of stability.

It has been said that glycerol solutions of trypsin are stable through long periods. On diluting such extracts with water we have found that they do not long preserve their activity. Even where sterilized water was used for the dilution, we found a marked decrease in action when the solutions stood overnight at room temperature. The change may be in part due to the comparative absence of inorganic matters in such dilutions. At a temperature of 40° these pure aqueous solutions suffered greatly.

Pancreatin solutions (F. B and F. as used above), however, when protected with toluene, were found to be fully active after standing 41 hours at room temperature, as shown by fibrin digestion.

In another experiment the effect of longer heating was determined.

Experiment M. 50 mg. portions of trypsin were incubated at 40° in a volume of 50 cc. of water through different periods, as shown below. Then to each flask 100 mg. of sodium carbonate were added and the incubation continued through three hours at the same temperature with the addition of 3 grams of moist fibrin which had been kept in chloroform water. Kjeldahl tests were finally made to determin the amount of digestion.

No.	Prelim. digest. Min.	Soluble N. Mg.	Residue N. Mg.	Total N. Mg.
1.....	30	48.4	49.0	97.4
2.....	45	50.4	..	..
3.....	60	53.5	42.6	96.1
4.....	90	42.6	..	..
5.....	120	47.5	50.4	97.9
6.....	180	42.8	54.9	97.7

It appears that at this temperature the effect of incubation is not much greater for a period of three hours than for half an hour. Some figures from a later experiment will suggest, however, that the best results are obtained by immediate digestion.

The Effect of the Amount of Alkali.

A few experiments only will be offered here, as we are concerned with the one alkalin substance only, sodium carbonate. It is usually stated that trypsin is not weakened or destroyed by moderate amounts of sodium carbonate, up to about 0.5%. This depends, however, on the temperature, as the following tests show. While elevation of temperature weakens the trypsin greatly, almost complete destruction follows if much alkali is present. In the following experiments portions of 100 mg. of pancreatin in a volume of 50 cc. were incubated with and without alkali through different times at temperatures of 40°, 50° and 60°. They were then brought to the same alkalinity and incubated with 3 grams of fibrin through periods, as shown, at 40°.

Experiment N. Preliminary incubation with and without alkali, using pancreatin (F. B. and F.) and 3 grams of pressed fibrin (N content 123.5 mg.).

No.	Prelim. alkali. Mg.	Prelim. inc.	Alkali added. Mg.	Final inc. Hrs.	Result.
1.....	...	1 hr. at 40°	100	6	Digestion completed
2.....	100	1 hr. at 40°	...	6	Digestion completed
3.....	...	1 hr. at 40° + 1 hr. at 50°	100	6	Two-thirds digested
4.....	100	1 hr. at 40° + 1 hr. at 50°	...	15	Slight digestion
5.....	...	1 hr. at 40° + 1 hr. at 50° + 1 hr. at 60°	100	15	No digestion
6.....	100	1 hr. at 40° + 1 hr. at 50° + 1 hr. at 60°	...	15	No digestion

In numbers 1 and 2 the time and alkali effects are but slight, as the digestion is complete in 6 hours. In number 3 the action of the higher temperature is shown in the preliminary digestion and when alkali is present at the outset the ferment seems to lose all its activity, practically, as shown in number 4. In numbers 5 and 6 the temperature alone seems to be sufficient to stop the digestion. These experiments were repeated a number of times with essentially the same results. Even when a smaller amount of fibrin was used and the stronger trypsin it was found that the prolonged heating with alkali above the temperature of 40° resulted in a marked weakening, or practical destruction of the ferment.

Finally, the effect of incubation with increasing amounts of carbonate was determined. The results show that the ferment is active in presence of a relatively large weight of this alkali.

Experiment O. 50 milligram portions of trypsin were incubated during 30 minutes with increasing amounts of carbonate, as shown. In three cases enough hydrochloric acid was then added to neutralize all but 100 mg. of the carbonate, leaving all of the same alkalinity as the first test. In the proper cases sodium chloride was added to make all alike in this respect, and after the addition of fibrin the incubation was continued through 2 hours at 40°. Kjeldahl tests were made on the filtrate and residue of the digestion.

In two of the numbers below, 5 and 6, there was no preliminary incubation with the carbonate. This was added with the fibrin at the beginning of the actual digestion. In the preliminary digestion the volume was 50 cc., and was made to 80 cc. in the final digestion in numbers 1, 2, 3 and 4. In 5 and 6 the volume was 50 cc.

No.	Prelim. alk. Mg.	Final alk. Mg.	Soluble N. Mg.	Residue N. Mg.	Total N. Mg.
1.....	100	100	77.1	22.7	99.8
2.....	200	100	79.4	22.1	101.6
3.....	300	100	84.6	16.0	100.6
4.....	500	100	85.7	16.0	101.7
5.....	...	100	98.0	4.5	102.5
6.....	...	500	98.0	4.2	102.2

The fact is brought out pretty clearly that in the preliminary incubation, the presence of the carbonate is protective of the ferment. It is also shown that where there is no preliminary incubation the final digestion is much nearer complete. Part of the apparent effect may, however, be due to the salt added, or formed in the other cases. The important fact which is shown is that the trypsin ferment exhibits the same activity in the presence of 1% of sodium carbonate that it exhibits with 0.2%. In practice digestions may be carried out within these limits with like results, as long as the temperature is no higher than here.

Résumé.

These investigations were undertaken to determine the behavior of trypsin in presence of acids, alkalies, and water through long contact, and the action of pepsin and acid on trypsin, points which are still in dispute, notwithstanding the numerous studies recorded in the literature.

Raw egg albumin is not a suitable substance to use in testing the proteolytic action of trypsin preparations, as it is scarcely digested by even the best tryptic ferments obtainable. Some of the experiments of Wroblewski, Bednarski and Wojczynski are rendered meaningless by this use of raw egg. Trypsin digests boiled egg but slowly, as compared with pepsin. For such experiments fibrin and casein are the best materials to employ for comparative studies.

Where it was desirable to use egg albumin we have employed with success an imported hen's egg albumin which comes from China. It was found to yield a very uniform coagulum, and gave a pretty constant nitrogen value when gram portions were weighed. Most dried albumins are unsuitable for such experiments.

Most of the trypsin preparations in the market are very weak, while some are quite inert. The pancreatins come in these groups. For scientific studies we have found the "trypsin" of Fairchild Brothers and Foster, New York, very satisfactory and useful.

In preliminary incubations of half an hour trypsin is not appreciably injured by an acidity of 0.32% hydrochloric acid, as shown by the power of digesting relatively large weights of fibrin after neutralization with alkali. In such tests we have taken three to five grams of fibrin as the basis for comparison, rather than a few milligrams. The conclusion cannot be drawn, however, that much digestion takes place in presence of acid. A trace of acid stops the digestion without destroying the ferment.

While trypsin is not destroyed or greatly weakened by the action of 0.2% hydrochloric acid, it is greatly weakened in presence of the acid and pepsin. But trypsin acting in neutral solution on pepsin does not appear to alter the activity of the latter. The fuller investigations on this part of the subject are to be reported later.

Aqueous solutions of trypsin are weakened by long standing at the

ordinary temperature, and the purer the product, the more rapid the deterioration. This has been found to be true in working with the dilutions of glycerol extracts of the pancreas with water. But in the incubation of trypsin solutions at 40° there does not appear to be much change between 30 minutes and 180 minutes. At a temperature of 50° the weakening is rapid, while at 60° destruction follows.

In agreement with the results of other workers, trypsin is active in the presence of rather large weights of sodium carbonate. A 1% concentration of this salt does not weaken the activity in fibrin digestion at 40°. This corresponds roughly to a hydroxyl strength of 0.01 normal at this temperature. At higher temperatures the action of the carbonate is much more marked, and here we find the effect of the water incubation hastened. In the digestion of fibrin by trypsin 0.2% and 1% sodium carbonate have about the same action.

In these experiments we have been assisted by Mr. George W. Muhleman, to whom we extend our thanks.

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STUDIES IN BACTERIAL METABOLISM XIII-XXX.

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Received June 26, 1913.

XIII. Certain Factors which Influence Bacterial Metabolism.

The chemistry of cellular metabolism is one of decided importance, but one in which the exact details are still largely unknown. The most important results bearing upon this subject up to the present time have been obtained from experiments upon man and the higher animals: in them, metabolic balances have been carried out with a great degree of precision, but the very complexity of the problem, including as it does modifications attributable to an extensive physiological division of labor between cells, tissues and organs, does not permit of more than a very generalized conception of the actual internal mechanism involved. Such experiments have measured the alpha and the omega of the process satisfactorily and furnished much collateral information as the experimental conditions are varied along definit lines: the true inter- and intra-cellular chemistry has not been elucidated up to the present time.

The bacteria offer rather unusual opportunities for the study of unicellular metabolism, for with them it is possible to subject cells of the same kind to careful measurements. The results obtained cannot as yet be applied *in toto* to the cells of the human body, but at least the general principles of cellular metabolism, which these unicellular organisms exhibit, have more than academic interest: they have a practical bearing