Second: That the turbidity is due to germination of the spores of the mould *Penicillium glaucum*, due to the action of glucose as a chemical stimulus.

Third: That the presence of alcohol and carbon dioxide have no appreciable effect on the appearance of the turbidity in glucose solutions.

Fourth: That maltose does not act as a chemical stimulus on spores of *Penicillium glaucum* and hence maltose solutions do not grow turbid on standing.

BLOOMINGTON, ILLINOIS.

[CONTRIBUTION FROM THE LABORATORY OF THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

ON THE ASSUMED DESTRUCTION OF TRYPSIN BY PEPSIN AND ACID. II.[1] OBSERVATIONS ON ANIMALS.

BY J. H. LONG AND MARY HULL.

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In our recent paper with the above title[2] we showed by a long series of experiments *in vitro* that the common proteolytic enzyme of the pancreas, isolated as trypsin, is capable of withstanding a rather long digestion in presence of hydrochloric acid and pepsin, provided sufficient protein of some form is present to combine with all or part of the acid and so bring the hydrogen-ion concentration down to a certain level. This behavior of protein is of great practical importance, and failure to recognize it led to much confusion in the study of the mutual action of certain enzymes. It was further brought out that trypsin must exhibit a certain degree of activity in solutions which have a faintly acid reaction, rather than the usual alkaline reaction. This is contrary to the long accepted notion that this enzyme is active in alkaline medium only, but many phenomena point to this relation.

In the subsequent prosecution of the work we have relied on evidence secured through experiments on animals, partly with dogs and partly with the human subject. Satisfactory methods were not immediately available. In the work with dogs our first attempts were in this direction. Duodenal fistulas were made in a number of animals at a point between the pylorus and the entrance of the main pancreatic duct. A bent glass canula introduced into this opening was provided with an expanded end and so turned that it would collect the chyme flow from the stomach, but would prevent the upward flow of the bile and pancreatic secretion. Working in this way it appeared possible to obtain the unmixed

[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.

[2] THIS JOURNAL, 38, 1620 (1916).

gastric contents. The dogs so treated were fed on prepared foods to which the desired amount of trypsin was added. After remaining in the stomach through a sufficient interval the partially digested mixtures were allowed to flow through the canula to be collected and examined for the persisting trypsin. But unfortunately no data of positive value could be obtained by this general scheme, because, as a rule, the animals could not be kept alive long enough to give results which might be considered normal. This theoretically simple plan was tried in consequence of the desire to eliminate at the start any complication arising from the presence of pancreatic trypsin, but the physiological difficulties in the way of securing a permanent fistula in the short space between the bile duct and the pylorus seem to be very great.[1]

There is this practical objection to the introduction of a duodenal fistula at a much lower point, along with the section or ligature of the pancreatic duct. The chyme secured in this way contains too much admixed bile, and besides there is always the possibility of the presence of erepsin to obscure the interpretation of the results of the tests on the chyme for trypsin.

As the prime object of the investigation was to determine the presence or absence of tryptic activity in mixtures of pepsin, acid and food, as such mixtures may exist in the stomach, it was finally decided to make the observations on the stomach itself, and in such a manner as to exclude or to allow for the interference from the regurgitated intestinal contents which might contain, possibly, some trypsin. To carry out a plan of this sort several methods of observation are available. We have begun by feeding certain definite test meals to dogs and after a time withdrawing the stomach contents as far as possible and examining for acidity, presence of bile as suggesting regurgitation, and peptic and tryptic activity. This was done first with the normal diet and was followed by a similar meal to which a known weight of active trypsin was added. The problem was to determine the existence of increased tryptic activity after the ingestion of the commercial enzyme.

Experiments on the Normal Stomach.

The trypsin preparations used in the tests were the same as described in our last paper, and had the strength there given. The usual test meal consisted of 16 g. of cracker crumbs with 200 cc. of water to which 50 or 100 g. of cooked and finely ground meat were added. To insure the ingestion of the whole of the food, or food and trypsin, the mixtures were fed through a tube and funnel and washed down with the last of the water. Occasionally some egg was added to the meal and the water was at times increased. After a lapse of 45 to 120 minutes from the time of feeding

[1] Our thanks are due to Dr. W. R. Cubbins for his kindness in making a number of duodenal fistulas for us in our efforts to utilize the method.

the tube was introduced and the remaining contents drawn off as rapidly as possible. The volume of the liquid collected was noted. A small fraction of this was used for tests for free and combined acid, and separate portions for pepsin and bile tests. It may be said here that in all cases examined a good pepsin reaction was obtained, which fact need not be mentioned in the tables. Bile was usually absent, and in such cases, when no trypsin was ingested with the food, a tryptic reaction was practically negative, or so slight as to be questionable. With bile present from regurgitation a trypsin test was usually positive, but weak.

The trypsin test was made on a larger aliquot part of the stomach contents and in this manner: 25 cc. (occasionally less) of the liquid was made neutral to phenolphthalein with sodium hydroxide and mixed with 20 cc. of a molar/15 phosphate solution having a P_H value of 7.7. A gram of powdered fibrin was added and shaken until soaked and swollen, after which the mixture was made to 50 cc. and incubated through 3 hours at 40°. A parallel blank test was always made on an equal volume of the stomach contents, but boiled to kill the ferment present. After the incubation the 50 cc. digested mixtures were chilled to stop action, poured into 100 cc. cylinders containing sufficient volume of barium hydroxide and chloride solution to precipitate all the phosphate present, and made up to the mark with water. The cylinders were allowed to stand overnight to permit the precipitates to settle, after which 50 cc. portions were poured or filtered off, neutralized to phenolphthalein and titrated with 0.2 *N* alkali in presence of formaldehyde in the usual manner. From the results of the titrations in the unboiled samples the blank results were subtracted. The differences multiplied by 2 gave the titration values for the original portions (25 cc. or less), measured from the stomach contents. The values for the whole contents were, in turn, calculated from these. In Table A the data, as so outlined, are given.

These tests were carried out over an extended period on one dog and over shorter periods on two other dogs. Many of the results were so near alike that they might be considered as duplicates. A large number of these are omitted from the table. The first column gives the date, the dog number and the amount of meat or egg added to the basic diet. In the next columns we have the weight of trypsin powder ingested and the time the mixture was allowed to remain in the stomach. The volume of withdrawn contents follows, and this is all that could be readily brought up by the tube. The next columns show the amount of free and combined acid present, calculated for the total volume. In some cases these values are but close approximations only because a sufficiently large amount for fuller tests could not be spared from what was needed for the trypsin tests. The presence or absence of bile is indicated in the next column and then the amount taken for each trypsin test, equal volumes being

employed in the direct and boiled tests. In the following columns the alkali required in each titration is shown and then the net result for the volume measured out, 2 (*a*—*b*). The difference between the two titrations is doubled because only half of the measured volume is actually used in each case. The last column gives the amino nitrogen liberated, calculated for the whole stomach content.

The results as a whole are interesting. When trypsin is not ingested the amount of nitrogen in amino combination liberated from the substrate fibrin is always minute, except when there is evidence from the appearance of bile that proteolytic ferment may have entered the stomach from the intestine. Even here the tryptic activity is not strong in any case. In one of the dogs the tendency to regurgitation was seldom noticed, while in another, No. I, it was rather common, as in man. It is safe to conclude from these experiments that when trypsin is not ingested the tryptic activity of the stomach is so slight as to be negligible for our purpose. The large number of experiments here included point clearly to this conclusion. It was thought desirable to secure the data over an extended period so as to be able to reach a fair degree of certainty and settle the question satisfactorily.

On the other hand when trypsin is ingested with the meal we have two conditions to consider. In the one case we have the test meal of cracker crumbs and water alone and in the other the addition of meat in relatively large amount. When meat was not fed the accumulation of free acid in the stomach was usually marked, amounting in one instance to 0.42%. With the meat present the acid was naturally found to be largely combined. In these we observed the highest tryptic activity in the aspirated liquid, and this in many cases was very marked. At first sight the recorded values would appear to be too high to be possible in a number of instances. For example, in the case of Dog III, on April 27th we note the liberation of 0.143 g. of amino nitrogen from a fibrin substrate, and this is in excess of what might be expected from the gram of fibrin. It must be kept in mind, however, that the action on the gram of fibrin is about one-seventh of this, from the small volume of contents used. The results in the last column, it must be remembered, are calculated for the whole contents.

In the case of Dog III, June 23rd, we find a high tryptic activity when no meat was fed and when the free hydrochloric acid had reached 0.06%, and also on June 29th when the free acidity had reached 0.42%, but ordinarily there is marked tryptic activity only in the presence of the protecting protein which holds the acid. The strongest evidence of tryptic digestive power is shown on June 12th and June 13th with Dog III when there was no free acid but plenty of combined acid in the contents still remaining in the stomach.

TABLE A.

				Trypsin. G.	Time in stomach.	Vol. of liquid drawn.	Free HCl.	Combined HCl.	Bile present.	Vol. taken for trypsin test.	Cc. N/5 NaOH used for original (a).	Cc. N/5 NaOH used for blank (b).	2 (a—b).	Indicated g. of NH_2. N for whole stomach content activity.
III.	Apr.	25	100 g. meat.	1	45	80	0.000	0.584	—	25	15.2	9.9	10.6	0.095
III.	Apr.	26	no meat....	0	45	30	0.033	0.550	—	10	0.8	0.6	0.4	0.003[1]
III.	Apr.	27	100 g. meat.	1	60	80	0.000	0.443	—	25	11.3	3.3	16.0	0.143
III.	May	1	60 g. egg....	1	60	60	0.000	0.219	—	20	4.6	2.1	5.0	0.042
III.	May	2	100 g. meat.	0.5	60	130	0.000	0.057	—	25	1.7	1.0	1.4	0.020[2]
III.	May	3	100 g. meat.	1	60	80	0.000	0.292	—	25	7.6	3.4	8.4	0.065
III.	May	4	no meat.....	0	60	90	0.261	0.033	—	25	4.1	4.0	0.2	0.001
III.	May	8	50 g. meat..	1	45	110	0.000	1.121	—	25	3.1	1.9	2.4	0.030
III.	May	9	100 g. meat.	1	75	29	0.000	0.084	—	10	3.0	0.9	4.2	0.034
III.	May	15	50 g. meat..	1	50	37	0.000	0.055	—	15	2.7	1.0	3.4	0.024
III.	May	17	100 g. meat.	1	60	32	0.000	0.108	—	15	4.2	2.1	4.2	0.025
III.	June	4	100 g. meat.	1	60	130	0.000	0.303	—	25	8.6	3.5	10.2	0.136
III.	June	8	no meat.....	1	45	110	0.000	0.160	—	25	4.1	2.5	3.2	0.089[3]
III.	June	12	100 g. meat.	1	60	95	0.000	0.206	—	20	11.6	4.2	14.8	0.197
III.	June	13	50 g. meat..	1	60	109	0.000	0.341	—	25	14.1	5.8	16.6	0.203
III.	June	23	no meat.....	1	45	190	0.111	0.138	—	25	5.9	2.8	6.2	0.132
III.	June	26	no meat.....	1	60	80	0.057	0.029	—	25	8.4	3.3	10.2	0.091
III.	June	28	no meat.....	1	60	43	0.000	0.015	—	20	5.1	3.1	4.0	0.024[4]
III.	June	29	no meat.....	1	60	44	0.184	0.158	—	20	9.0	5.2	7.6	0.047
III.	July	10	100 g. meat.	0	75	92	0.000	0.168	—	30	3.1	3.1	0.0	0.000
III.	July	11	50 g. meat..	0	90	100	0.000	0.282	—	40	2.3	2.0	0.6	0.004
III.	July	12	50 g. meat..	0	120	56	0.000	0.036	—	25	1.2	1.2	0.0	0.000[5]
III.	July	13	50 g. meat..	0	75	48	0.000	0.052	+	20	2.6	2.0	1.2	0.007
III.	July	14	50 g. meat..	0	60	73	0.000	0.105	—	30	2.0	2.0	0.0	0.000
III.	Oct.	18	no meat.....	0	45	170	0.171	0.122	—	25	2.9	2.9	0.0	0.000
III.	Oct.	19	no meat.....	0	45	102	0.064	0.149	+	20	6.7	3.2	7.0	0.098
III.	Oct.	20	no meat.....	0	50	85	0.433	0.047	—	20	0.9	0.9	0.0	0.00

I.[1]	Oct.	16	no meat.....	0	45	100	0.250	0.086	—	20	2.7	2.7	0.0	0.000
I.	Oct.	17	no meat....	0	50	80	0.134	0.056	+	20	1.4	1.0	0.8	0.008
I.	Oct.	18	no meat.....	0	45	125	0.069	0.069	+	20	2.9	2.5	0.8	0.014
I.	Oct.	19	no meat....	0	45	150	0.075	0.255	+	20	4.7	2.6	4.2	0.085
I.	Oct.	20	no meat....	0	50	150	0.765	0.105	—	20	1.2	1.1	0.2	0.005
II.	Oct.	16	no meat....	0	45	150	0.327	0.225	—	20	2.1	2.1	0.0	0.000
II.	Oct.	17	no meat.....	0	50	160	0.080	0.138	—	20	1.0	0.9	0.2	0.004
II.	Oct.	18	no meat.....	0	45	130	0.234	0.065	—	20	1.6	1.6	0.0	0.000
II.	Oct.	19	no meat.....	0	45	110	0.088	0.154	—	20	1.6	1.6	0.0	0.000
II.	Oct.	20	no meat....	0	50	100	0.150	0.130	—	20	1.1	1.1	0.0	0.000
III.	Oct.	23	50 g. meat..	0	50	50	0.082	0.116	—	20	1.7	1.6	0.2	0.001
III.	Oct.	24	100 g. meat.	0	55	75	0.109	0.218	—	25	2.7	2.7	0.0	0.000
III.	Oct.	25	100 g. meat.	0	50	60	0.063	0.235	+	25	4.5	4.0	1.0	0.007
III.	Oct.	26	100 g. meat.	0	55	112	0.033	0.547	—	25	1.9	1.9	0.0	0.000
III.	Oct.	27	50 g. meat..	0	55	108	0.086	0.353	—	25	2.0	2.0	0.0	0.000
I.	Oct.	23	50 g. meat..	0	45	100	0.000	0.480	+	25	2.4	1.3	2.2	0.025
I.	Oct.	24	100 g. meat.	0	55	59	0.119	0.177	—	25	2.3	2.2	0.2	0.001
I.	Oct.	25	100 g. meat.	0	50	72	0.014	0.321	+	25	4.0	2.9	2.2	0.018
I.	Oct.	26	100 g. meat.	0	45	126	0.000	0.726	—	25	1.9	1.9	0.0	0.000
I.	Oct.	27	50 g. meat..	0	55	115	0.000	0.529	+	25	2.1	1.6	1.0	0.013
II.	Oct.	23	50 g. meat..	0	45	90	0.164	0.369	+	25	3.5	2.5	2.0	0.020
II.	Oct.	24	100 g. meat.	0	55	100	0.236	0.136	—	25	1.8	1.8	0.0	0.000
II.	Oct.	25	100 g. meat.	0	50	100	0.137	0.309	—	25	2.5	2.5	0.0	0.000
II.	Oct.	26	100 g. meat.	0	45	120	0.086	0.546	—	25	2.1	2.1	0.0	0.000
II.	Oct.	27	50 g. meat..	0	50	95	0.181	0.167	—	25	2.1	2.1	0.0	0.000

[1] Stomach was washed out before meal was given.

[2] The dog drank more water after the test meal.

[3] Note that with no meat given there is no free acid in the stomach contents.

[4] Not much left in stomach except mucus-like liquid.

[5] Time in stomach too long. Gave water and aspirated this as the remaining liquid was but little.

It will be noticed that the times given for the reaction in the stomach were, ordinarily, 45 to 60 minutes. More could not well be allowed with dogs as the contents pass rather rapidly into the duodenum, and even in the intervals chosen by us there was undoubtedly a considerable loss of contents in many cases. Taking the experiments as a whole, however, there is evidence that a good part of the proteolytic power of the administered trypsin persisted after this prolonged contact with acid and pepsin.

Experiments after Ligation of Pylorus.

We next carried out a number of experiments on two dogs in which the pylorus had been completely ligated so as to make a closed pouch of the stomach. After the abdominal operation to reach the organ the animals had been kept long enough in good condition to restore them to normal health and activity before the ligature was made.

Dog I.—After washing out the stomach by means of the tube a gram of trypsin was administered in a soft capsule and the flow of secretion stimulated by an injection of gastrin. After three hours 20 cc. of a light colored liquid were drawn off. representing nearly the whole contents. No free acid was recognized but some was held combined by the abundance of mucus present.

Trypsin tests were made on portions of 8 cc. by the methods already described.

In direct test used 6.1 cc. of 0.2 *N* NaOH. In blank, 3.7 cc. of 0.2 *N* NaOH. Digestion increase, 2.4 cc.

The amino acid nitrogen liberated by the trypsin remaining in the 20 cc. of liquid would therefore amount to 0.037 g., indicating the persistence of some of the trypsin through three hours. Other experiments on this dog were lost on account of repeated vomiting.

Dog II.—After washing the stomach the dog received a gram of trypsin and a gastrin injection. The animal was kept quite at rest through three hours and vomiting avoided. At the end of the time 80 cc. of contents were withdrawn by the tube. In aliquot parts free and combined acid were determined, giving for the whole volume 0.086 g. of free HCl and 0.029 g. of combined acid. Trypsin tests were made on 25 cc. portions with 1 g. of the fibrin, as before. By the formaldehyde titration we found:

In direct test used 6.5 cc. of 0.2 *N* NaOH. In blank, 3.5 cc. of 0.2 *N* NaOH. Digestion increase, 3.0 cc.

These figures correspond to a liberation of 0.054 g. of amino acid N for the whole volume of contents.

With the same dog 24 hours later, after washing the stomach, a new trial was made, feeding 1 g. of trypsin and following with the gastrin. After waiting three hours a volume of 72 cc. of contents was brought up by the tube. The free HCl found was 0.157 and the combined 0.052 g. For 25 cc. portions we found by the formaldehyde titration:

In direct test used 5.7 cc. of 0.2 *N* NaOH. In blank, 3.1 cc. of 0.2 *N* NaOH. Digestion increase, 2.6 cc.

This points to a liberation of 0.042 g. of amino N for the whole volume.

On the following day, that is, 48 hours from the beginning of the experiment, the dog was still in good condition for continued trials. The stomach was washed out and gastrin injected. A meal consisting of 10 g. of cooked meat, an egg, some water and a gram of trypsin was given. The dog was kept quiet through three hours and then the stomach content consisting of 60 cc. of acid liquid was withdrawn. The total free acid amounted to 0.273 g. and the combined acid to 0.224 g. or about 3.5% of the weight of dry protein ingested. A marked flow of acid seemed to be secured by the combined action of the gastrin and the food protein and the excess of free acid in the liquid amounted to about 0.45% of the weight of the 60 cc. collected. A test for trypsin was made here as before, but in this case the formaldehyde titration gave no evidence of the liberation of amino acid nitrogen. It was apparent that the ferment had been destroyed by the acid and pepsin secreted.

The results of these experiments are very interesting. Here no trypsin could work back from the duodenum, yet we find a good reaction in the case of Dog I and in two of the trials with Dog II. In these three instances the accumulation of free acid seemed to be too small to inhibit the tryptic activity. For Dog I no free acid was found, while for the other animal it was a trifle over 0.1% on the first day and about 0.2% on the second, calculated for the whole liquid volume. Many earlier observations from this laboratory go to show that trypsin withstands pretty well a degree of acidity not greater than this in the absence of much pepsin. On the third day, however, the acidity was markedly high, although not unusual in the dog, and here we find that the ferment was all destroyed, or so much weakened as to give no positive test. The conditions for the activation of pepsin by acid to the point where it will destroy trypsin, as shown by experiments *in vitro*, are indicated in our last paper.[1] Not much tryptic activity should be expected in any of the above experiments. That any at all is found in presence of the acid and pepsin, indicated by the qualitative tests made, points to a rather marked degree of tryptic persistence.

Experiments on a Dog with a Gastric Fistula.

In this series we employed one dog throughout, Dog VI on our list. An operation was made in March on an animal in good condition. The wound healed perfectly and in the course of about three weeks the animal appeared perfectly normal, with good appetite. The pylorus was not touched and the flow remained unobstructed.

A diet shown in the table attached, Table B, was fed along with variable

[1] THIS JOURNAL, 38, 1620 (1916).

amounts of water. The animal was kept in a hammock after the feeding until the time of observation was completed. Then the liquid remaining in the stomach was drawn off for tests. By waiting too long we frequently found that practically everything had left the stomach. In some of these experiments, as in others, the flow of an increased amount of juice was stimulated by the injection of gastrin. In the tests a good pepsin reaction was always noted, but bile was generally absent. No column is kept for these tests in the table, but the few times when bile was observed is stated in footnotes, as this may suggest regurgitated trypsin. The methods of testing employed were the same as in the previous cases. See Table B.

TABLE B.

			Trypsin given.	Time in stomach.	Vol. of liquid drawn.	Free HCl.	Combined HCl.	Vol. taken for trypsin test.	Cc. *N*/5 NaOH used for original, (*a*).	Cc. *N*/5 NaOH used for blank, (*b*).	2 (*a*—*b*).	Indicated g. of NH_2. N for activity of whole content.
May	2	no food	0	60	15	0.048	trace	5	1.0	1.0	0.0	0.000[1]
May	2	meat and milk	1	60	50	0.055	0.091	5	2.3	1.1	2.4	0.067
May	3	80 g. meat	1	60	25	0.000	0.081	5	2.9	1.5	2.8	0.039
May	4	50 g. meat, 1 egg	0	120	80	0.000	0.451	25	3.7	3.7	0.0	0.000
May	8	100 g. raw meat	0	90	12	0.000	0.018	3	0.6	0.3	0.6	0.007[2]
May	9	water only	0	45	13	0.000	0.002	5	0.6	0.4	0.4	0.002[3]
May	15	20 g. meat	0	50	8	0.000	0.017	3	2.1	2.1	0.0	0.000
May	16	50 g. meat, 2 eggs	1	60	172	0.000	0.940	25	4.2	3.1	2.2	0.042
May	17	50 g. meat, 1 egg	1	90	150	0.000	0.573	25	5.0	4.1	1.8	0.030
May	24	50 g. meat, 1 egg	1	60	90	0.049	0.115	25	5.6	3.6	4.0	0.040
June	1	no food, gastrin	0	90	16	0.043	trace	5	0.2	0.2	0.0	0.000
June	4	no food, gastrin	0	120	37	0.215	trace	5	0.2	0 2	0.0	0.000
June	7	no food, gastrin	1	30	14	0.079	0.000	6	1.5	1.0	1.0	0.007
June	8	no food, gastrin	1	30	9	0.048	0.000	3	3.1	1.9	2.4	0.020[4]
June	12	50 g. meat, 1 egg	1	60	105	0.063	0.471	25	6.6	5.0	3.2	0.038
June	13	50 g. meat, 2 eggs	1	120	75	0.066	0.200	25	9.8	5.0	9.6	0.081[5]
June	15	50 g. meat, 2 eggs	1	120	61	0.178	0.266	25	8.1	5.2	5.8	0.040[6]
June	19	50 g. meat, 2 eggs	1	120	83	0.046	0.175	25	7.4	4.9	5.0	0.047
June	21	50 g. meat, 1 egg	1	75	52	0.058	0.116	20	8.0	3.9	8.2	0.060
June	23	2 eggs, much water	1	60	260	0.000	0.286	25	5.2	2.5	5.4	0.157
June	26	50 g. meat	1	105	16	0.035	0.018	7	2.1	2.1	0.0	0.000
June	28	50 g. meat	1	60	75	0.164	0.410	25	16.7	7.6	18.2	0.163
June	29	1 egg	1	60	100	0.000	0.218	20	4.6	2.0	5.2	0.073
July	11	50 g. meat, 2 eggs	0	120	75	0.027	0.216	30	4.8	4.8	0.0	0.000
July	12	50 g. meat, 1 egg	0	120	56	0.010	0.101	20	4.6	4.6	0.0	0.000
July	13	50 g. meat, 1 egg	0	60	67	0.034	0.194	25	3.0	3.0	0.0	0.000

[1] Initial juice after washing out the stomach.

[2] A little bile was present and possibly trypsin from regurgitation.

[3] Bile present. The slight tryptic action may be due to duodenal contents.

[4] Note persistence of some tryptic action in presence of 0.5 % HCl.

[5] Note tryptic action after 2 hours.

[6] Same point in this and the following experiment.

The evidence for the persistence of trypsin in the presence of pepsin and acid is as clear from these experiments as from the earlier ones. When trypsin was not administered it was not found in the stomach contents except in traces accompanying bile from the duodenum. In only a few instances of administration do we fail to find it in the stomach fluid and then only when the time elapsed was long enough to allow the stomach material to go into the duodenum or when the acid secretion was pretty high. Such a case appears on June 26th, when the time was long and the remaining contents small in volume but high in percentage acidity. On June 7th and 8th the acid content of the liquid brought up was about 0.5%. Such a concentration in presence of pepsin is usually speedily destructive, and there was no protecting protein here. The highest residual tryptic activity is noted on June 23rd and June 28th when the time was an hour. Free acid was absent in one case but was rather high in the other.

It will be noted that the figures for liberated amino acid nitrogen appear rather low in many instances. In general, however, they represent a good degree of tryptic activity because in every case a large part of the stomach contents and added trypsin had undoubtedly passed unhindered into the duodenum. The liquid volumes secured for the tests simply represent what had not escaped in this manner.

Reactions in a Pawlow Pouch.

Finally we took up the problem of the behavior of trypsin in contact with acid and pepsin from another angle. To avoid entirely the complication from the possible presence of duodenal trypsin in the stomach, as a consequence of regurgitation, we have made use of a false stomach or Pawlow pouch in a dog.[1] Our first plan of procedure was to place in this pouch small amounts of liquid and protein plus the trypsin for observation. A good secretion of acid and pepsin was always secured in the pouch and the action of this on the trypsin and protein was observed after a time. But the plan was not satisfactory for observations of any length as the secreted liquid could not be retained in the pouch except when the dog was kept on its side or in some other uncomfortable position. Finally this scheme was adopted: The secreted juice was allowed to flow as fast as formed from the false stomach into a small bottle held in a container strapped to the body of the animal. At the outset the trypsin, when used, and a constant weight of cooked and finely ground meat, 5 g., were placed in the bottle which was held in the proper position through three hours, the dog being supported in a hammock meanwhile. In this manner the bottle constituted a third stomach which was maintained

[1] Our thanks are due to Dr. W. Keeton of the Department of Pharmacology for the work of this operation and for much other valuable help during the progress of the investigation.

at the body temperature by the circulation of water in the container outside. Besides being bound tightly to the body of the animal, the metallic jacket was surrounded by pads to assist in retaining the right warmth.

Under these conditions a considerable flow of a normal gastric juice took place into the glass stomach, which had to some extent the motions of the dog's abdomen. At the beginning of the experiment the animal was fed in the usual way and generally received 200 g. of meat and an egg. The larger flow of the gastric juice naturally went to the normal stomach but there was always an appreciable amount in the other direction to be collected finally in the bottle. Table C gives the results of the tests made on the bottle contents. The 5 g. of meat were usually well digested, pointing to the activity of the collected juice. The tests for tryptic action in the resulting liquid were made as before on a substrate of 1 g. of fibrin in the phosphate mixture.

The figures here reported seem to show a rather high degree of proteolytic activity in the trypsin remaining in the bottle after having been subjected to the influence of the pepsin and the hydrochloric acid. The substrate for the final test was the gram of fibrin, and the amino acid N reported is more than could be furnished by that weight of protein. But the same explanation holds here as before. The values in the last column show the nitrogen which would be liberated from the whole of the liquid collected in the bottle, while the gram of fibrin is acted upon by a fraction only of this volume. It must be recognized, in addition, that the substrate is in reality more than the fibrin since the meat in the glass stomach has been largely liquefied in the operation and must be attacked also by the trypsin. In some cases as much as 2 g. of meat go in this way to form part of the substrate.

In a few cases the combined hydrochloric acid calculated for the whole flow appears very high. The collecting bottle contains the protein of the trypsin as well as the meat, but the latter has generally undergone partial digestion, leaving substances which have a higher binding power than the original protein. It is also to be noted that the acid secreted was often in a state of partial combination with mucus or some other protein-holding substance. This is illustrated in the collection of July 11th, for example, where neither meat nor trypsin was put in the bottle. In any event the secretion of acid in this dog must be considered as relatively large.

Giving due weight to all the factors in this case it is still evident that the trypsin has exhibited a marked resisting power to the combined action of the acid and pepsin in the presence of protein. The large number of tests recorded show that the results are not accidental. While rather marked differences in the extent of the fibrin digestion are recognized, there is always some action and usually decided where unkilled trypsin

TABLE C.

	Trypsin in jar.	Juice collected. Cc.	Free HCl.	Combined HCl.	Vol. for trypsin test.	Cc. N/5 NaOH for original, (a).	Cc. N/5 NaOH for blank, (b).	2 (a—b).	Indicated g. of NH_3 N for activity of whole collection.
April 27.....	1.0	50	0.000	0.232	20	21.5	6.0	31.0	0.217
May 1.....	1.0	25	0.000	0.139	10	16.6	5.6	22.0	0.154
May 2.....	0.5	31	0.000	0.112	10	13.7	4.5	18.4	0.160
May 3.....	0.5	22	0.000	0.120	10	13.5	4.5	18.0	0.111
May 4.....	0.0	25	0.105	0.011	10	2.3	2.3	0.0	0.000[1]
May 8.....	0.5	37	0.027	0.377	10	2.8	2.6	0.4	0.004[2]
May 9.....	0.5	34	0.000	0.172	10	3.2	3.0	0.4	0.003[2]
May 15.....	0.5	42	0.061	0.305	10	2.6	2.5	0.2	0.002[2]
May 16.....	1.0	58	0.042	0.190	10	4.1	4.0	0.2	0.003[2]
May 22.....	1.0	56	0.000	0.184	25	4.0	4.0	0.0	0.000[2]
May 24.....	1.0	55	0.040	0.460	25	15.4	7.4	16.0	0.099
May 31.....	1.0	41	0.000	0.447	15	13.0	5.6	14.8	0.113
June 1.....	1.0	40	0.000	0.364	15	14.1	7.4	13.4	0.100
June 4.....	1.0	45	0.066	0.426	20	14.2	5.6	17.2	0.108
June 6.....	1.0	50	0.027	0.473	20	15.9	5.9	20.0	0.140
June 7.....	1.0	46	0.000	0.218	20	12.3	5.9	12.8	0.082
June 8.....	1.0	50	0.021	0.291	20	13.0	6.0	14.0	0.098
June 12.....	1.0	44	0.016	0.321	20	17.0	4.4	25.2	0.165
June 13.....	1.0	50	0.013	0.418	20	13.9	6.0	15.8	0.110
June 15.....	1.0	53	0.058	0.270	20	14.9	7.1	15.6	0.116
June 19.....	1.0	35	0.013	0.078	15	14.2	5.4	17.6	0.115
June 21.....	1.0	45	0.031	0.106	20	18.2	6.9	22.6	0.142
June 23.....	1.0	51	0.074	0.158	20	10.3	1.9	16.8	0.120
June 26.....	1.0	45	0.033	0.131	20	12.3	7.6	9.4	0.059
June 27.....	1.0	57	0.031	0.207	25	11.4	5.3	12.2	0.077
June 28.....	1.0	50	0.018	0.180	20	11.4	4.6	13.6	0.095
July 11.....	0.0	41	0.056	0.128	15	3.0	3.0	0.0	0.000[3]
July 12.....	0.0	50	0.273	0.018	20	1.9	1.9	0.0	0.000[3]
July 13.....	0.0	45	0.147	0.006	15	1.3	1.3	0.0	0.000[3]

was used. In addition to this there is some evidence suggesting that the trypsin may even aid in the digestion and liquefaction of the meat placed in the bottle. This is the case where the free acid is rather low, below the point where it is capable of inhibiting the proteolytic action of the pancreas enzyme. It is not likely that pepsin and trypsin can act simultaneously as the hydrogen-ion concentration which makes peptic digestion possible, is above the level where tryptic action is appreciable or possible.

[1] Juice collected through 1 hour only.

[2] In all these cases the trypsin was killed by heat before being added to the bottle. Note the high combined acid in the collection of May 8th and on several of the following dates.

[3] In these cases no meat was placed in the collecting bottle, as well as no trypsin. The low results of the titrations probably point to the presence of protein in the secreted juice, as is suggested by the high combined acid noted in some cases.

But with a denatured protein like cooked meat, trypsin may act before the hydrochloric acid has accumulated to the inhibiting point, or after it has become largely combined with the split products.

In the formaldehyde titrations the so-called blanks are high in most cases. Here we have the groups originally present or which are formed by the peptic digestion in the preliminary stage. The tests of May 4th, 8th, 9th, 15th, 16th and 22nd disclose low blanks because either no trypsin or inactive trypsin was present. On the other dates the much higher blanks suggest that we must have some little splitting of amino bonds even in the preliminary or acid stage. These blanks are approximately twice as high as in the no-trypsin cases and are most satisfactorily explained on this hypothesis. The collections of April 27th, May 24th, June 1st, June 15th, June 21st and June 26th are good illustrations. When the other tables are analyzed some similar cases are found.

Conclusions.

In these experiments carried out on dogs to determine the combined effect of hydrochloric acid and pepsin on trypsin, under conditions which correspond to those obtaining in the human stomach at times, when the latter ferment is ingested, four lines of observations were followed.

In these four groups of observations, after the ingestion of trypsin, the stomach contents were secured (*a*) by means of a tube after the ligation of the pylorus, in which case the organ constituted a closed pouch in which the secretion followed normally for a time, (*b*) from the normal open stomach by the tube applied at the proper interval after the ingestion of food and added trypsin, (*c*) by means of a gastric fistula made in the normal organ and opened from time to time for the withdrawal of contents, and (*d*) from a false stomach or Pawlow pouch constructed from the normal organ.

In all the animals the secretion of pepsin and acid was abundant, and from this point of view the conditions for the persistence of trypsin were not favorable. Yet, in the larger number of experiments, this latter ferment was not destroyed by the other combination where sufficient protein was present to bring the concentration of the free acid down to a certain value. Trypsin seemed to be destroyed or greatly weakened only when the acid was in excess with pepsin.

These experiments appear to confirm our earlier conclusions from work done *in vitro* that trypsin, pepsin and hydrochloric acid may exist side by side under conditions which, following the ingestion of trypsin, may exist in the human stomach. It is even possible that some trypsin proteolysis may occur then in that organ when the free acid is very low from protein combination. The destruction or weakening of the trypsin is a function, probably, of the hydrogen-ion concentration.

CHICAGO, ILL.