It has been shown that a urea-splitting enzyme is present in normal gastric juice (1). Its range of activity was at least between pH 1.4 and 8.3, and its greatest action was found to take place at pH 7.8. Its activity was slow, and continued over a space of several days; the greatest part had taken place by the end of the first 24 hours. Owing to these characteristics it was suggested that the enzyme was probably different from that isolated and crystallized from the soy bean by Sumner (2).

In connection with the studies described in Paper I (3), it became of interest to determine to which of the two protein or protein-like substances of the gastric juice the urea-splitting enzyme and pepsin were attached.

**EXPERIMENTAL**

Unless specified, methods of procedure were identical to those used in Paper I (3).

**Determination of Urea-Splitting Enzyme**

*Not Dialyzed*—Mixtures of gastric juice containing free HCl were adjusted to various hydrogen ion concentrations from 1.4 to 6.3. After the contents of the tubes were made equal with water, they were one-half saturated with magnesium sulfate. The contents of the tubes were filtered through Whatman No. 42 filter paper, and the precipitate was redissolved in an amount of water equal to the original gastric juice specimen. Filtrates and redissolved precipitates were analyzed for nitrogen content. 10 cc.
portions of the contents of each tube were adjusted to pH 7.4 with
phosphate buffer, and 2 cc. of a solution containing 7.04 mg. of
urea nitrogen per 100 cc. were added to the filtrate. The original
urea content of the filtrate was 2.52 mg. of nitrogen per 100 cc.
To 10 cc. of the redissolved precipitate buffered as above, 2 cc. of a

<table>
<thead>
<tr>
<th>Adjusted pH of gastric juice</th>
<th>Approximate pH of tubes after one-half saturation with MgSO₄</th>
<th>Protein N per 100 cc. gastric juice</th>
<th>NH₃-N after incubation with urea, 1.53 mg. per cent</th>
<th>Protein N per 100 cc. gastric juice</th>
<th>NH₃-N after incubation with urea, 1.53 mg. per cent</th>
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<td>6.50</td>
<td>17.81</td>
<td>0.10</td>
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</tbody>
</table>

NH₃-N from gastric juice buffered and incubated at pH 7.4 = 0.2
" " gastroglobulin " " " " 7.4 = 0.0
" " urea " " " " 7.4 = 0.0
" " gastric juice " " " stored in ice box 7.4 = 0.0

solution containing 9.16 mg. of urea nitrogen per 100 cc. were
added. This made a final urea nitrogen concentration of 1.53 mg.
per cent in each tube of filtrate and precipitate, for at this time
there were two series of tubes, one of filtrate and one of precipitate,
which contained urea. To obtain controls, the contents of the
tubes were halved. In this way it was possible to place similar
preparations of each tube in the incubator at 37.5° and in the ice
box at 7.0° for 48 hours. At the end of this period the contents of the tubes were analyzed for ammonia nitrogen, urea nitrogen, and ammonia and urea nitrogen in toto, as described in a previous article (1). The results are shown in Table I.

Dialyzed—A second series of tubes was identically arranged and one-half saturated with magnesium sulfate. After filtration the precipitates were redissolved in an amount of water equal to the amount of gastric juice from which they were obtained. Filtrate and precipitate were dialyzed in celloidin membranes prepared by the method of Pierce, as previously described (3). Dialysis was continued until the dialysate was found free from sulfates. As shown in Paper I, only the gastroglobulin nitrogen remained within the sacs.

The contents of the sacs were made equal with water, and a portion of each was analyzed for nitrogen. Another portion representing a known amount of nitrogen was adjusted to pH 7.4 with phosphate buffer. 2 cc. of a solution containing 9.16 mg. of urea nitrogen per 100 cc. were added to each tube and the contents were brought to uniform volume, making a final urea nitrogen concentration of 1.53 mg. per cent. Toluene was added to each tube. The contents of each tube were halved; one part was incubated at 37.5° for 48 hours; the other was placed in the ice box, temperature 7.0°. At the end of this period the specimens were analyzed for ammonia nitrogen, urea nitrogen, and total ammonia and urea nitrogen, as previously described (1). The increase of ammonia nitrogen was quantitatively accounted for by the decrease in urea nitrogen. Controls were run with (a) the original gastric juice incubated without the addition of urea, (b) gastroglobulin incubated with addition of urea (a portion of this material was incubated without urea), (c) urea blank, (d) the original gastric juice which had been placed in the ice box.

In a previous article (1) it has been shown that ammonia production occurred without demonstrable presence of bacteria. Mixtures were cultured before and after incubation at 37.5°.

The results of a single experiment which were typical of the three carried out are shown in Table I.

An analysis of the data demonstrates several points. (1) The non-dialyzed urea-splitting enzyme was found in connection with the gastroglobulin and its greatest activity was slightly greater
than the activity of the original gastric juice. (2) The amount of ammonia formed was in direct relation to the amount of gastroglobulin. (3) After dialysis the urea-splitting enzyme tended to increase in activity and it was again in direct relation to the amount of gastroglobulin. (4) No ammonia was formed in any of the tubes placed in the ice box or where gastroglobulin or urea was

<table>
<thead>
<tr>
<th>Adjusted pH of gastric juice</th>
<th>Approximate pH of tubes after one-half saturation with MgSO₄</th>
<th>Not dialysed</th>
<th>Dialysed</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Protein N in mg. per 100 cc. gastric juice</td>
<td>Non-protein N after incubation 1 hr. at 37.5°C with 1 per cent casein at pH 1.4</td>
<td>Protein N in mg. per 100 cc. gastric juice</td>
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<td>Precipitate*</td>
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<td>6.50</td>
<td>17.81</td>
</tr>
</tbody>
</table>

Non-protein N from peptic activity in gastric juice = 21.2 mg. per 100 cc.  
" " casein control = 0.0 " " 100 "*

* Not determined in filtrate owing to heavy MgSO₄ precipitate.

incubated separately. (5) The amounts of ammonia nitrogen formed and urea nitrogen destroyed were approximately equal.

**Determination of Pepsin**

A similar study was made on the enzyme pepsin. Peptic activity was measured by the amount of non-protein nitrogen
formed from a 2 per cent casein suspension, which had been mixed
with 2 cc. of a 1:10 dilution of gastric juice or of an equal amount
and dilution of the various filtrates and precipitates. The mix-
tures were incubated for $\frac{1}{2}$ hour in a water bath at 38°. The unit
of measurement was expressed as mg. of non-protein nitrogen
formed by the action of 100 cc. of original juice acting for $\frac{1}{2}$ hour
on 2 per cent casein. Proteins were precipitated by the addition
of 5 cc. of 20 per cent trichloroacetic acid. Nitrogen of the filtrate
was determined by the method of Folin and Wu (4).

Peptic activity was determined on dialyzed and non-dialyzed
materials similar to those used for the investigation of the urea-
splitting enzyme activity. Owing to the large amounts of MgSO$_4$
in the non-dialyzed filtrate a determination could not be carried
out on this portion.

The data are shown in Table II and demonstrate the following
points: (1) Active pepsin was found only in connection with the
gastroglobulin. (2) The activity of the dialyzed and non-
dialyzed enzyme was in direct relation to the amount of gastro-
globulin. (3) The peptic activity of the specimen precipitated
at pH 3.5 was practically equal to that of the original gastric juice.

**DISCUSSION**

From the work reported it is shown that pepsin and a urea-split-
ting enzyme were found in close quantitative relation to the gastro-
globulin of the gastric juice; so close in fact that one wonders if the
enzymes do not form most, if not all, of the protein.

Various investigators have shown that the isoelectric point of
pepsin obtained from the gastric mucosa is about pH 3.0. Nor-
throp has shown that the pepsin which he has crystallized has pH
2.75 as its isoelectric point. The gastroglobulin described above
has its isoelectric point at pH 3.5.

**SUMMARY**

Data have been presented to show that a urea-splitting enzyme
and pepsin are quantitatively related to the gastroglobulin of the
gastric juice. The urease activity was extremely weak and not
comparable with that found in soy bean extract or in some bacte-
rial cultures.
BIBLIOGRAPHY

GASTRIC JUICE: II. STUDIES ON A UREA-SPLITTING ENZYME AND PEP SIN IN RELATION TO THE PROTEINS
Lay Martin


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