7540 P

# Studies on Enzymatic Digestion of Gastric Mucin.

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Although it is generally assumed that mucin is digested in the intestinal tract, no systematic investigations on the enzymatic hydrolysis of mucin have been reported. We have, therefore, undertaken to study the action of enzymes upon mucin *in vitro* for the purpose of determining its possible manner of cleavage.

The general procedure consisted in adding a known amount of enzyme to a mucin solution of known concentration at a pH well within the range of activity of the enzyme being tested. Controls consisted of mucin alone and enzyme alone at approximately the same pH. At intervals, usually 2 days, samples were removed and analyzed, until no further breakdown beyond that occurring in the controls could be detected. Proteolysis was followed by the nitrous acid method of Van Slyke.[1] Cleavage of the carbohydrate portion of the molecule was tested for by determining the presence or absence of reducing substances, using the Somogyi-Shaffer-Hartmann method.[2] The extent of cleavage was also determined by the decrease in the amount of mucin precipitable in 70% alcohol, and subsequent analysis of the precipitated material. The activity of all enzymes used was demonstrated by their hydrolysis of suitable substrates. In those cases in which digestion of mucin was observed, a second portion of enzyme was subsequently added to preclude the possibility that cessation of hydrolysis was due to depletion or inactivation of the enzyme.

We have previously reported the isolation of a purified mucin from commercial preparations by a process of peptic digestion followed by 70% alcohol precipitation.[3] Since peptic digestion is used in the preparation of commercial mucin, the additional peptic digestion was employed for the sole purpose of rendering alcohol soluble any extraneous protein material present as a result of improper manufacture, or subsequently added as a diluent. In the

[1] VanSlyke, D. D., *J. Biol. Chem.*, 1929, **83**, 425.

[2] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Baltimore, 1932, **2**, 469.

[3] Anderson, R. K., Fogelson, S. J., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 518.

absence of these conditions, identical material can be obtained by alcohol precipitation, either with or without additional peptic digestion. The mucin used in these investigations was prepared from an undiluted commercial preparation by precipitation in 70% alcohol. Mucin prepared in this manner was entirely resistant to further peptic action. It is quite possible that commercial mucin represents merely the peptic resistant portion of native mucin, though no conclusive evidence is available on this point.

In studying the action of trypsin upon mucin, enzyme preparations from 3 different commercial sources were employed. One sample was tested and contained, in addition to trypsin, the ereptic enzymes dipeptidase and aminopolypeptidase. The data obtained with all 3 preparations were practically identical, the amounts of amino nitrogen liberated being equal to 7.57%, 7.90%, and 7.85% of the total nitrogen of the mucin.

The commercial erepsin employed was found to contain, in addition to erepsin, a small amount of proteinase. When added to a tryptic digestate of mucin, the additional amino nitrogen liberated was equal to approximately 3% of the total nitrogen, or a total increase of approximately 11% due to the combined action of trypsin and erepsin.

Table I summarizes the results when cleavage was followed by means of 70% alcohol precipitation and subsequent analysis of the precipitated material. These data, in agreement with those obtained by the Van Slyke method, indicate partial digestion by both trypsin and erepsin.

TABLE I.

%Precipitation, Nitrogen and Reduction of Mucin Digestates.

| | % Precip.* | % N. | % Reduction† (as glucose) |
|---|---|---|---|
| Before digestion | 83.2 | 7.15 | 34.5 |
| After tryptic digestion | 76.5 | 6.9 | 38.2 |
| Tryptic + ereptic digestion | 73.4 | 6.2 | 44.2 |

* By alcohol at 70% concentration.

† After acid hydrolysis.

Proteolytic enzymes were also prepared from yeast by water extraction and their action upon mucin tested. An increase in amino nitrogen equal to 15.1% of the total nitrogen was observed.

The following enzymes were tested with negative results: maltase (alpha glucosidase), emulsin (beta glucosidase), steapsin and pancreatic amylase.

An intestinal extract was prepared by water extraction of the duodenum and first few inches of the ileum of a dog. The extent of digestion was practically identical with that caused by combined action of commercial trypsin and erepsin. The addition of these enzymes produced no further cleavage. No digestion of the carbohydrate portion of the molecule was observed.

These studies indicate that mucin, in contrast to most proteins, is relatively resistant to enzymatic hydrolysis *in vitro*, a property in accord with its ascribed protective action. However, it seems probable that further digestion of mucin occurs in the digestive tract. This is indicated by the fact that normally no readily detectable quantities of mucin are excreted from the intestinal tract, and, furthermore, that glucuronic acid of mucin is available for conjugation.[4] Perhaps specific enzymes exist for this purpose, or some definite but at present unknown sequence of enzymatic action is required. These studies are being continued with purified enzyme preparations.

## 7541 C

## Depressor Extracts of Some Human Tissues.*

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The recent work of Dale, Dudley, and their associates[1, 2, 3] has thrown new light on the depressor substances that may be extracted from animal tissues. The methods of preparation of and differentiation between the various depressor substances have been described especially by Chang and Gaddum.[1] Because these methods are rather new, they have not yet been applied extensively to human tissues. The present work was undertaken to determine if there were any unusual amount of depressor substance in (1) carcinomatous tissue and in (2) toxic thyroid tissue.

All specimens were obtained from living patients during a sterile surgical operation. The specimens were extracted in 3 hours or less

[4] Miller, C. O., Brazda, F. G., and Elliott, E. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 633. Miller, C. O., and Connor, J. A., *Ibid.*, 1933, **30**, 630.

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[1] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[2] Dudley, H. W., *J. Physiol.*, 1933, **79**, 249.

[3] Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **72**, 74.