THE ESTIMATION OF MUCIN IN GASTRIC JUICE*

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The importance of mucin as a constituent of gastric mucus and of acid
gastric juice has been generally recognized (2). Numerous methods for
the quantitative determination of mucin have been described, the estima-
tion of the reducing power having been the method almost universally
used. The viscosity, acid-combining power, nitrogen content, and iodine-
combining power have each been considered as a measure of mucin con-
centration; methods based on these properties have been applied to the
whole gastric secretion or its contents or to various fractions isolated by
ultrafiltration, electrodialysis, and the use of various protein precipitants
(3–8). However, none of these methods has gained general acceptance (2).

Mucotinsulfuric acid is regarded as a characteristic prosthetic group of
gastric mucin and many other mucoproteins (9). It has been isolated
from hog gastric mucus (10–13) and from pure canine gastric juice (14).
Hexuronic acid (12) or, more specifically, glucuronic acid (9, 13) has been
established as one of the four components of this polysaccharide. Since
(gluc)uronic acid appears to be the most characteristic component of
gastric mucin and several reliable methods for its estimation are available,
we investigated the possibility of utilizing the estimation of glucuronic
acid as a measure of the concentration of mucin in gastric juice. We have
found that a modification of Tollens' naphthoresorcinol reaction for glu-
curonic acid (15) results in a consistent recovery of uronic acid from gastric
mucin, mucotinsulfuric acid, and whole canine gastric juice or mucus.

EXPERIMENTAL.

The conclusions and opinions expressed in these studies are based on the
results of more than 2800 determinations made in experiments of various
types over a period of more than 2 years. The experiments presented here
in the form of various tables and figures were selected on the basis of their
illustrative value.

Preliminary Experiments

In our preliminary experiments we used the procedure outlined by
Maughan, Evelyn, and Browne (16) for the determination of glucuronic

* This paper was read at the Fifty-sixth annual meeting of the American Phys-
iological Society in Chicago, May, 1947 (1).
acid derivatives in urine as being the least complicated quantitative procedure of the reported modifications of Tollens' naphthoresorcinol reaction. When applied to gastric mucin, mucoitinsulfuric acid, and pure canine gastric juice, it produced a color characterized by the maximal absorption of light transmitted by Rubicon Filter 565. It is well known, however, particularly from the studies of Levene and his associates (9), that rather prolonged hydrolysis with strong acid is required for complete degradation of mucoitinsulfuric acids. Therefore it was necessary to establish conditions of hydrolysis which would result in the optimal recovery of uronic acid from mucin. Boiling for varying lengths of time with approximately 3 N hydrochloric acid in the presence of naphthoresorcinol was used by many investigators for the colorimetric estimation of uronic acids and their various derivatives (17-19). Similarly, boiling with comparable concentrations of hydrochloric acid was found to be successful in effecting hydrolysis of mucoitinsulfuric acid and chondroitinsulfuric acid with the liberation of their monosaccharide components. Accordingly, it was desirable to establish the optimal conditions for the hydrolysis of mucin compatible with the optimal development of color with naphthoresorcinol.

The sources of various preparations employed in this study were as follows:

The glucuronic acid was obtained from the A. D. Mackay Company, New York, and had a warranted purity of less than 5 per cent lactose and a melting point of 146° (uncorrected).

The menthylglucuronic acid was isolated from the urine of menthol-fed rabbits by the method of Williams (20). After the material had been purified by crystallizing it thrice, the melting point was 92.1° (uncorrected).

The gastric mucin was a preparation previously described by one of us (14). It was isolated from 15.4 liters of pure gastric juice obtained from dogs equipped with a gastric fistula and esophagotomy. The ash content was 0.50 per cent, and the elementary composition calculated for the ash-free substance was C 52.68 per cent, H 7.00 per cent, N (Dumas) 14.02 per cent, S (in the form of ethereal sulfate) 0.372 per cent, and P 0.00 per cent. The reducing power (Hagedorn-Jensen method after hydrolysis with N HCl for 6 hours at 100° in a sealed tube) was equivalent to 14.67 per cent glucose. The barium salt of mucoitinsulfuric acid was isolated in a high state of purity in a yield of 4.32 per cent. In all the experiments the preparation of gastric mucin mentioned above was dissolved in 0.02 N sodium hydroxide.

The mucoitinsulfuric acid (acid sodium salt) was isolated from Wilson's "gastric mucin" by the method of Levene and López-Suárez (11) and had an ash content of 4.97 per cent. The elementary composition (calculated
for the ash-free substance) was C 41.23 per cent, H 6.20 per cent, N (Dumas) 5.31 per cent, S (in the form of ethereal sulfate) 1.65 per cent, and P 0.00 per cent. The reducing power (Hagedorn-Jensen method after hydrolysis with N HCl for 6 hours at 100° in a sealed tube) was equivalent to 61.8 per cent of glucose. While this preparation was not of a high degree of purity, it compared favorably with those described by Levene (9). Aqueous solutions of this substance were used in all the experiments.

First we studied the effect of extended boiling without otherwise modifying the procedure of Maughan et al. Some of these results are illustrated in Fig. 1 (section A). If the boiling was continued for 4½ hours, the optical density of the chromogen when measured with Filter 565 increased in a rather regular manner in the case of all the substances investigated, but there was a definite lag in the color development in the case of mucosulfuric acid as compared with glucuronic acid. That there was a relative lag also with mucin is evident from the fact that, while the optical density after 30 minutes of boiling was practically identical with that for glucuronic acid, there was a far greater development of color with mucin than with glucuronic acid or menthyl glucuronide. All curves tended to level off when boiling was continued for 3½ to 4½ hours. In a number of other experiments in which boiling was limited to ½ to 1 hour, the lag in the color development with mucin was even more pronounced than in the experiments illustrated in Fig. 1. This phenomenon is unquestionably due to the fact that glucuronic acid as such is immediately available for the formation of chromogen, but when it is a constituent of mucin it must first be liberated in a free state. The behavior of menthyl glucuronide was very similar to that of glucuronic acid probably because it may be hydrolyzed with relative ease. The leveling off of the color development, observed with all the above substances when boiling is extended for 3½ hours or more, can be explained by the fact that the chromogen formed with glucuronic acid and naphthoresorcinol under the conditions of our experiments reaches its maximum at about 4 hours. In this respect our observations confirm the earlier observations of Kapp (17) and Hanson et al. (19). Therefore it might be expected that with a sufficiently extended boiling time there should be no material difference in chromogen formation regardless of whether the glucuronic acid is available immediately as in the case of free glucuronic acid or whether it is only gradually liberated from mucin, provided the latter process is accomplished in a relatively short period of time.

The practical conclusions to be drawn from the above observations are that true recoveries of glucuronic acid may be expected with the procedure of Maughan et al., if boiling is extended to 4 hours or more, or that a shorter procedure might perhaps be developed if a certain degree of preliminary
hydrolysis of mucin preceded the "coupling" with naphthoresorcinol. In order to study the latter possibility, another series of experiments was carried out, in which the aforementioned substances were subjected to preliminary hydrolysis with 3 N HCl in a boiling water bath for 270 minutes, this being followed by "coupling" with naphthoresorcinol for a fixed period of 30 minutes. This procedure will be further referred to as "separate" hydrolysis and coupling, as distinct from the term "simultaneous" hydrolysis and coupling referred to in the experiments already described. The results of the "separate" hydrolysis and coupling experi-

![Fig. 1. Color development in the naphthoresorcinol reaction under various conditions of hydrolysis and coupling. A, Curve 1, gastric mucin (100 mg. per 100 ml.); Curve 2, glucuronic acid (1 mg. per 100 ml.); Curve 3, menthylglucuronic acid (2 mg. per 100 ml.); Curve 4, mucoitin sulfuric acid (10 mg. per 100 ml.). In B, the time includes 30 minutes coupling.](http://www.jbc.org/)

ments are illustrated in Fig. 1 (section B). A progressive fall in color intensity with glucuronic acid and menthyl glucuronide occurred in these experiments, as was to be expected in view of the well known fact that glucuronic acid is rather easily destroyed by boiling with strong hydrochloric acid. Similar relations occurred with mucoitin sulfurous acid, while the curve for mucin tended to rise till the end of 60 minutes and then to remain more or less constant.

When the recovery of glucuronic acid from mucin and mucoitin sulfurous acid was calculated from the light densities in each of the experiments graphically illustrated in Fig. 1, the following results were obtained. In
"simultaneous" hydrolysis and coupling experiments the recovery of glucuronic acid from mucin was considerably higher with 60 minutes than with 30 minutes boiling, and there was some tendency to a further increase if heating was extended further. The recovery of uronic acid from mucotinsulfuric acid after 30 minutes of "simultaneous" hydrolysis and coupling was not uniform, but after 60 minutes it was uniform and not materially increased when the time of hydrolysis was extended. In experiments in which mucin and mucotinsulfuric acid were subjected to preliminary "separate" hydrolysis, there was a steep increase in glucuronic acid recovery, especially from mucin. However, this should be regarded as only an apparent effect, due to the more rapid destruction of free glucuronic acid in the standards than in uronic acid, which is gradually liberated from mucin.

### Table I

Estimates of Glucuronic Acid Content (Per Cent) in Mucotinsulfuric Acid and Mucin under Various Conditions of Hydrolysis and Coupling

<table>
<thead>
<tr>
<th></th>
<th>Simultaneous hydrolysis and coupling, Filter 565</th>
<th>30 min. hydrolysis followed by 30 min. coupling</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min. 60 min. 240 min. Filter 565 Filter 565 and 400</td>
<td></td>
</tr>
<tr>
<td>Mucoitinsulfuric acid</td>
<td>11.6 ± 0.6* 9.4 ± 0.4 6.94 ± 1.08 11.0 ± 0.2 12.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Mucin</td>
<td>1.08 ± 0.04 1.21 ± 0.06 1.63 ± 0.36 1.34 ± 0.04 1.28 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

The above figures in each case represent the results of six determinations which were started simultaneously with the same solutions.

* Standard deviation = ±√Σd²/(n - 1) (Fisher).

These experiments therefore indicated that extended "separate" hydrolysis could not well be employed because of the deterioration of the standards. The highest recoveries of glucuronic acid from mucin and mucotinsulfuric acid were obtained with the 30, 60, and 240 minute "simultaneous" procedure and also with the 30 minute preliminary "separate" hydrolysis. These procedures were subjected to more detailed study in order to determine more exactly the magnitude and also the reproducibility of the uronic acid recovery.

The results of a representative experiment are shown in Table I. The highest mean recovery of glucuronic acid from mucin was obtained with the "simultaneous" procedure of 4 hours duration, but the recoveries were not consistent (coefficient of variation 22 per cent). The next highest recoveries which were at the same time coincident with the smallest deviations (coefficient of variation 4 per cent) occurred when the mucin solu-
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Tions were subjected to separate hydrolysis for 30 minutes prior to the 30 minutes "coupling."

This method, as will be demonstrated below, produced reliable results when applied to gastric mucin and pure canine gastric juice, but was found to be less satisfactory when applied to alkaline or neutral mucus. The hydrolysis of alkaline or neutral mucus with hydrochloric acid gave rise to large amounts of furfural, which interfered with the development of color with naphthoresorcinol. However, furfural and glucuronic acid produce entirely different colors, as may be seen from the absorption spectra (Fig. 5). Maximum absorption in the visible spectrum in the case of furfural takes place in the range of light transmitted by Rubicon Filter 400 and in the case of glucuronic acid in the band transmitted by Filter 565. Straight line calibration curves were obtained for the light densities measured at these two wave-lengths for both of these substances. Therefore conditions are present which permit corrections for extraneous furfural by application of the principles suggested by Knudson et al. (21) for a two-component color system. With calculations based on this principle, described below under "Method," more satisfactory recoveries were obtained from alkaline mucus and from gastric juice containing considerable proportions of mucus.

Method

Reagents—

2. Naphthoresorcinol. 0.2 per cent filtered (No. 42 Whatman filter paper) aqueous solution; must be prepared immediately before analysis.
3. Ether. Merck, reagent, treated with 1 per cent ferrous sulfate to remove peroxides, washed with water until sulfate-free, and stored over anhydrous sodium sulfate.
4. Ethyl alcohol. 95 per cent.
5. Standard solutions of menthy glucuronic acid and glucuronic acid. A stock standard solution of menthy glucuronic acid is prepared so as to contain 4 mg. per ml., which may be kept in the refrigerator for not more than a month. A dilute standard solution (1:100) is made up at the time of analysis. The standard solution of glucuronic acid, containing 0.02 mg. per ml., must be prepared immediately before analysis.

Procedure

Hydrolysis—A sample of material (gastric juice or solution of mucin), containing 0.01 to 0.6 mg. of uronic acid (2 ml. for histamine gastric juice,

1 Naphthoresorcinol was obtained from the Schwarz Laboratories, Inc., 202 East 44th Street, New York 17, New York.
1 ml. for sham feeding juice, and 0.2 ml. for mucus), is pipetted into special calibrated colorimeter tubes, and the volume is adjusted with distilled water to 2 ml. Tubes with 2 ml. of a standard solution and 2 ml. of water (blank) are set up simultaneously. 1 ml. of concentrated hydrochloric acid is added, the contents being thoroughly mixed, and each tube is covered with a glass marble and placed in a boiling water bath for 30 minutes.

Coupling—The tubes are removed from the water bath, 2 ml. of naphthoresorcinol solution are added to each, and the tubes are thoroughly shaken. 1 ml. of concentrated hydrochloric acid is added. The contents of the tubes are mixed and the tubes are covered and placed again in the boiling water bath for a further period of 30 minutes.

Chromogen Extraction—The tubes are withdrawn and cooled in an ice bath for 10 minutes. 2 ml. of ethyl alcohol are added to the contents and mixed, followed by 15 ml. of ether. The tubes are stoppered with rubber stoppers and shaken well by continuous, vigorous inversions for 30 seconds. The contents of the tubes are allowed to settle for 10 minutes, and the upper purplish colored layer is read in a special tube holder in the Evelyn colorimeter, with Filter 565 for the single filter procedure and Filters 565 and 400 for the two-filter procedure, after the blank (reagent) tube has been set at 100. The center setting should be no higher than 78. If it is any higher, the experiment must be discarded. As a rule the excessive color is due to deterioration of the naphthoresorcinol.

Calculations—For the one-filter procedure, \( \frac{L_{565}^u}{L_{565}^z} \) = mg. of glucuronic acid per 100 ml. of material if 0.02 mg. of glucuronic acid is used as standard and 2 ml. of material are taken.

For the two-filter procedure,

\[
\frac{K'_{565} \cdot L'_{400} - K'_{400} \cdot L'_{565}}{K'_{565} \cdot K_{400} - K'_{400} \cdot K_{565}} \times \frac{100}{V} = \text{mg. glucuronic acid per 100 ml. material}
\]

where \( V \) = ml. of material taken, or

\[
\frac{0.86 \cdot L'_{400} - 2.70 \cdot L'_{565}}{-11.8} \times 50 = \text{mg. glucuronic acid per 100 ml. material}
\]


These stoppers must be first thoroughly washed with acetone until the washings are colorless. Before and after each set of determinations they are adequately rinsed with ether. These stoppers are used for these determinations exclusively. The suitability of any particular batch of stoppers is best indicated by the “center setting” of the blanks and the reproducibility of the calibration constants of the standard solutions.
if 2 ml. of material are taken and the calibration constants given below are used.

\[ L_{400}^{\text{unknown}} = \text{optical density of unknown with Filter 400} \]
\[ L_{565}^{\text{unknown}} = \text{“ “ “ “ “ “ 565} \]
\[ L_{565}^{\text{standard}} = \text{“ “ “ standard “ “ 565} \]

**Calibration Constants**—\( K = \frac{\text{optical density}}{\text{(mg. per aliquot)}} \). With Filter 565, \( K_{565} = 0.86 \) for furfural and \( K_{565} = 4.72 \) for glucuronic acid. With Filter 400, \( K_{400} = 2.70 \) for furfural and \( K_{400} = 1.09 \) for glucuronic acid.

### Table II

<table>
<thead>
<tr>
<th>Filter No.</th>
<th>Substance</th>
<th>( n )</th>
<th>Calibration constants*</th>
<th>Glucuronic acid</th>
<th>Coefficient of variation</th>
<th>Coefficient of variation</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>565</td>
<td>Glucuronic acid</td>
<td>22</td>
<td>5.30, 0.45</td>
<td>8.5</td>
<td>102.0†, 4.5</td>
<td>4.4</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Menthylglucuronic acid</td>
<td>218</td>
<td>2.82, 0.15</td>
<td>5.3</td>
<td>13.3, 1.7</td>
<td>12.8</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Mucoidinsulfuric acid</td>
<td>45</td>
<td>0.69, 0.09</td>
<td>13.0</td>
<td>1.37, 0.09</td>
<td>6.0</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Mucoitinsulfate</td>
<td>29</td>
<td>0.067, 0.006</td>
<td>8.8</td>
<td>2.63, 0.02</td>
<td>0.8</td>
<td>_</td>
</tr>
<tr>
<td>565 and 400</td>
<td>Canine gastric juice</td>
<td>18‡</td>
<td>0.30, 0.02</td>
<td>6.0</td>
<td>13.6, 1.2</td>
<td>8.8</td>
<td>_</td>
</tr>
<tr>
<td>565 and 400</td>
<td>Mucoidinsulfuric acid</td>
<td>45</td>
<td>1.22, 0.00</td>
<td>7.4</td>
<td>1.22, 0.00</td>
<td>7.4</td>
<td>_</td>
</tr>
</tbody>
</table>

* \( K = \frac{\text{optical density}}{\text{(mg. or ml. per aliquot)}} \); s.d. = \( \pm \sqrt{\frac{\sum d^2}{(n - 1)}} \); coefficient of variation = (s.d./mean) \( \times 100 \).
† From twenty-two determinations.
‡ Triplicate determinations of six dilutions of the same specimen, obtained after sham feeding from a dog with a gastric fistula and esophagotomy.

Since no preparations of gastric mucin of generally accepted purity, which could be employed as a standard, were described, the conversion of glucuronic acid values to those of mucin cannot be justified. However, in some of our studies (22), when such a conversion was deemed necessary for clarity of presentation, a conversion factor derived from the mean values of glucuronic acid content in our best preparation of mucin presented in Table II was used.

**Absorption Spectra and Recovery Curves**

Using the procedure described above, we have compared the absorption spectra of gastric mucin, mucoidinsulfuric acid, glucuronic acid, menthyl-glucuronic acid, furfural, and gastric juice of different degrees of purity.
From Fig. 2 it may be seen that the absorption curves for glucuronic acid and its derivatives and for pure canine gastric juice, such as that obtained, for example, at the height of the secretion produced by sham feeding, are identical, maximum absorption with Filter 565, while the curve for furfural (Fig. 5) is utterly different. These observations justify the use of single Filter 565 for pure gastric juice. The color development of the above reference substances and pure gastric juice follows Beer’s law for optical densities measured with Filter 565 (Fig. 3). Straight line curves for glucuronic acid recovery for gastric mucin and mucoitin sulfuric acid were always obtained with the one-filter procedure, as illustrated by Fig. 4. The recovery of glucuronic acid in the experiments in which mucin was added to canine gastric juice was equally satisfactory.

Reliability of Method

The reproducibility of the one-filter procedure may be considered adequate in view of the values for standard deviations and coefficients of variation (Table II). In experiments performed over a period of 2 years in a routine manner with different batches of reagents and with a wide range of concentrations of all the substances studied, the coefficient of variation ranged from 5.6 to 12.8 per cent. Much greater uniformity was obtained in individual experiments even on a very large scale, as may be seen from Table I (last section), where the coefficients of variation for
ESTIMATION OF MUCIN

Fig. 3. Calibration curves with one-filter procedure in terms of optical densities.

Fig. 4. Calibration curves with two-filter procedure in terms of the estimated glucuronic acid content.
our routine procedure were only 1.8 per cent for mucoitinsulfuric acid and 3.0 per cent for mucin.

The absorption curves obtained with gastric juice which is not quite pure, particularly if mixed with mucus, have characteristics common to both glucuronic acid and furfural (Fig. 5). Many specimens of mucus (Fig. 5, Curve 5), especially those obtained from rats, and the first specimens of acid gastric juice collected in experiments on gastric fistula dogs (Fig. 5, Curve 4), which always contain a considerable amount of admixed mucus, give absorption curves closely resembling those of furfural. It was for such specimens that we found it necessary to use our two-filter

![Graph](http://www.jbc.org/)

**Fig. 5.** Comparison of the light absorption curves of various types of gastric secretion with those of glucuronic acid and furfural.

procedure. With mucin and mucoitinsulfuric acid, the reproducibility of the two-filter procedure was comparable to that obtained with the one-filter modification, as may be seen from the values for standard deviations and coefficients of variation in Table II. There was a slight difference in the magnitude of glucuronic acid recovery, but this does not seem to be significant. Straight line glucuronic acid recovery curves for mucin and mucoitinsulfuric acid resulted with both modifications (Fig. 4). Comparable recoveries with both modifications were obtained in experiments in which known amounts of mucin were added to canine gastric juice. However, the available evidence seems to indicate that truer recoveries
of mucin are obtained from alkaline gastric mucin and from not quite pure gastric juice with the two-filter than with the one-filter procedure. However, this evidence is not conclusive and further study is necessary.

Source of Uronic Acid in Gastric Juice

Mucoprotein should in all probability be regarded as practically the only source of the uronic acid that is liberated on acid hydrolysis of pure canine gastric juice. This is evident from the results of experiments in which uronic acid was determined in the filtrates after the removal of protein by several procedures; viz., precipitation with acetone, basic lead acetate, and aluminum hydroxide. Acetone, under the experimental conditions obtaining, has been shown to precipitate all protein from freshly secreted canine gastric juice (23); precipitation with lead acetate at pH 6.4 to 6.8 has been generally regarded as one of the few specific precipitation procedures for mucoproteins, and we have found that aluminum hydroxide precipitates both the pepsin and the mucin of gastric juice quantitatively. Results obtained with gastric juice secreted in response to sham feeding are shown in Table III. Not more than 15 per cent, and in the majority of these experiments only 2 to 5 per cent, of the total glucuronic acid was recovered from these filtrates. Virtually all the glucuronic acid of the gastric juice (with a mean of 98.4 per cent) was recovered after the crystalloids had been removed by overnight dialysis. Similar results were obtained in several dialysis experiments with alkaline mucus collected from dogs with a gastric fistula and esophagotomy.

It is known that pepsin in an acid medium slowly digests mucoproteins, and peptic digestion has in fact been extensively used in the past as a preliminary step in isolating the carbohydrate complex of mucoproteins, especially chondroitinsulfuric acid. In our experiments with protein precipitants, the specimens of gastric juice were subjected to analysis not immediately after collection but after standing at 5° for 3 to 24 hours. Some degree of hydrolysis may therefore have taken place with the splitting off of mucosulfuric acid and its derivatives, and this may account for the small amounts of glucuronic acid found in the deproteinized filtrates. Therefore it is justifiable to conclude that the uronic acid of the gastric juice is derived predominantly or perhaps even exclusively from its protein constituents.

Mucin Content of Gastric Secretion under Different Conditions of Stimulation

The method described above was instrumental in establishing several physiologically important facts concerning the quantitative aspects of mucin secretion in relation to the nature of the stimulation. This part of
the study will be published in detail elsewhere (24). However, several observations should be stressed here. Table IV shows that there were exceedingly wide variations in the mucin concentration of different types

### Table III

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>pH</th>
<th>Chloride</th>
<th>Pepsin, Mкт</th>
<th>Total glucuronic acid</th>
<th>Non-dialyzable fraction*</th>
<th>In filtrates after precipitation with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m. g. per l.</td>
<td>mg. per 100 ml.</td>
<td></td>
<td></td>
<td>Lead sub-acetate</td>
</tr>
<tr>
<td>1</td>
<td>0.78</td>
<td>160</td>
<td>85</td>
<td>2.90</td>
<td>89 (8)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>2</td>
<td>0.96</td>
<td>154</td>
<td>31</td>
<td>1.56</td>
<td></td>
<td>10 (2)</td>
</tr>
<tr>
<td>3</td>
<td>0.91</td>
<td>167</td>
<td>92</td>
<td>1.44</td>
<td></td>
<td>2 (2)</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>164</td>
<td>98</td>
<td>1.35</td>
<td>90 (12)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>5</td>
<td>0.98</td>
<td>165</td>
<td>117</td>
<td>0.98</td>
<td>115 (4)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>6</td>
<td>0.94</td>
<td>171</td>
<td>41</td>
<td>0.68</td>
<td></td>
<td>0 (2)</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>172</td>
<td>23</td>
<td>0.61</td>
<td></td>
<td>0 (2)</td>
</tr>
</tbody>
</table>

* Expressed in percentage of total glucuronic acid.

The figures in parentheses represent the number of experiments with respective specimens.

Gastric juice or mucus was introduced into cellophane tubing (Nojax, Visking Corporation) and allowed to dialyze against tap water, distilled water, or physiological saline.

For precipitation with lead subacetate (Merck) the "free" acid in the gastric juice was neutralized with a calculated amount of 1.0 N NaOH, and 0.1 N NaOH was added to make the pH 6.8 to 7.0. 10 per cent lead subacetate was then added from a burette until no further precipitation was obtained. After standing in the refrigerator overnight, the sample was centrifuged and filtered. The pH of the filtrate ranged from 6.4 to 6.8.

For precipitation with colloidal aluminum hydroxide two procedures were used which gave comparable results. (1) The "free" acidity of the aliquot of gastric juice was neutralized with a calculated amount of N NaOH, and 0.1 to 0.2 volume of colloidal aluminum hydroxide was added. The solution was left in the refrigerator overnight and the supernatant was then filtered through No. 40 Whatman filter paper.

Precipitation with acetone was carried out as previously described (23).
obtained after sham feeding were in the middle range. These wide variations are important methodologically, since (a) they should be considered in selecting the size of the aliquots to be taken for mucin determination, and (b) they indicate that the magnitude of error inherent in the method described is sufficiently small not to jeopardize its value in physiological studies.

Table IV
Mucin Concentration (As Glucuronic Acid) in Gastric Secretion under Various Conditions of Stimulation

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Dog</th>
<th>Type of secretion</th>
<th>Rate</th>
<th>pH</th>
<th>Pepsin Mett units</th>
<th>Glucuronic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing</td>
<td>A</td>
<td>Fasting</td>
<td>0.53</td>
<td>8.02</td>
<td>0</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sham feeding</td>
<td>12.5</td>
<td>1.08</td>
<td>58</td>
<td>2.6*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot; and histamine</td>
<td>12.5</td>
<td>0.94</td>
<td>3</td>
<td>0.34*</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Spontaneous mucus</td>
<td>0.52</td>
<td>8.20</td>
<td>0</td>
<td>7.56</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Sham feeding, total secretion</td>
<td>0.3</td>
<td>1.03</td>
<td>33</td>
<td>1.84*</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Sham feeding, height of secretion</td>
<td>10.2</td>
<td>0.93</td>
<td>31</td>
<td>0.61*</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>After histamine</td>
<td>14.0</td>
<td>0.92</td>
<td>&lt;1</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>Sham feeding, total secretion</td>
<td>&gt;8.8</td>
<td>0.92</td>
<td>85</td>
<td>1.50*</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Spontaneous mucus</td>
<td>0.26</td>
<td>7.80</td>
<td>0</td>
<td>6.75</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>+ atropine</td>
<td>0.14</td>
<td>8.30</td>
<td>0</td>
<td>28.6</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>After sodium dodecyl sulfate</td>
<td>4.6</td>
<td>7.70</td>
<td>0</td>
<td>13.4</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>+ atropine</td>
<td>6.6</td>
<td>8.70</td>
<td>0</td>
<td>23.5</td>
</tr>
</tbody>
</table>

* Mucin in solution.

DISCUSSION

Glucuronic acid is regarded as a component of mucoitinsulfuric acid, the characteristic prosthetic group of gastric mucin (9, 13). Consequently we selected glucuronic acid or menthyl glucuronide or both as reference substances and expressed the results of our analyses in terms of glucuronic acid. The absorption curves obtained by us for glucuronic acid or its derivatives were quite different from those which we obtained for furfural. This provides further confirmation of the view, first expressed by Mandel...
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and Neuberg (25), that furfural is not responsible for the characteristic with HCl, as has been postulated by Tollens and many other investigators. The absorption curves produced by mucin and by pure gastric juice were strikingly similar to the curves produced by glucuronic acid, while those for alkaline gastric mucus showed features common to the curves for both glucuronic acid and furfural. This indicates that any method based on the determination of furfural alone cannot be utilized for the estimation of mucin. Furthermore, furfural formed from sources other than mucin was found to be detrimental to the estimation of mucoproteins by the naphthoresorcinol method if the calculations were based solely on the light density read at the band of maximum absorption with Filter 565. Only correcting for extraneous furfural made possible by application of the two-filter procedure seemed to make the naphthoresorcinol method more specific and to result in satisfactory recoveries from pure and not too heavily contaminated gastric juice.

We are aware that the method proposed here for the estimation of mucin in gastric juice does not meet the most exacting requirements of quantitative analysis, but to our knowledge it is the only method described so far which has been subjected to an exhaustive series of tests of reliability and has given results reproducible within 10 per cent. We believe that this method will be valuable in the solution of many important problems in the physiology and pathology of the gastric glands.

SUMMARY

A quantitative method for the estimation of mucin in the gastric juice and gastric contents has been developed, based upon the determination of glucuronic acid, a characteristic component of the prosthetic group of mucoproteins. The uronic acid is determined by a photoelectric-colorimetric method by the use of Tollens’ naphthoresorcinol reaction, as modified by Maughan, Evelyn, and Browne (16), after preliminary acid hydrolysis of the material. With gastric mucin and its derivatives the resulting color is a two-component color system with two maxima of light absorption obtained with Filters 565 and 400. The former band is characteristic for uronic acid itself and the latter for furfural, which may be derived either from uronic acid or from other substances, as in the case of gastric mucus or not quite pure gastric juice.

Reproducible results were obtained for mucin, mucoitin sulfuric acid, and pure gastric juice from the light densities determined in an Evelyn photoelectric colorimeter with Filter 565 alone, glucuronic acid or menthyl glucuronide being used as a standard. For mucus and contaminated gastric juice, it was necessary to determine light densities with Filters 565 and 400 by calculations based on the principles developed by Knudson,
ESTIMATION OF MUCIN

Meloche, and Juday (21). This procedure gave reproducible results also with mucin and mucoitinsulfuric acid. Fractionation experiments with various protein precipitants and dialysis demonstrated that only insignificant amounts of uronic acid were present in the protein-free fractions of canine gastric juice. It is probable that these small quantities may be derived from products of the enzymatic hydrolysis of mucin.

The concentration of mucin varied greatly in different types of gastric secretion. It was highest in alkaline mucus secreted either spontaneously or in response to intragastric instillation of sodium dodecyl sulfate and lowest in gastric secretion provoked by histamine administration. The concentration of mucin in the juice secreted in response to sham feeding was much higher than that of the gastric juice following histamine administration.

Addendum—Recently, after this study had been virtually completed, a new and specific color reaction for hexuronic acid with carbazole was reported by Dische (26), who claimed it to be suitable for the quantitative determination of hexuronic acid in various uronides and possibly also in some biological fluids. However, the presence of excessive amounts of protein in proportion to uronic acid appeared to jeopardize the results. We have attempted to explore the possibility of the application of this reaction to the determination of mucin in gastric secretion. The color development with glucuronic acid and menthylglucuronic acid (1 and 2 mg. per cent), when measured by the Evelyn colorimeter with Rubicon light Filter 520, was reproducible with a 12 per cent coefficient of variation, and the recovery of glucuronic acid from menthyl glucuronide was within 1 per cent of the theoretical value. The absorption spectra produced with our preparations of mucoitinsulfuric acid and gastric mucin were identical with that of glucuronic acid. A straight line recovery curve for hexuronic acid was obtained in experiments with gastric mucin (in a range of 100 to 400 mg. per cent) and mucoitinsulfuric acid (in a range of 3.0 to 50 mg. per cent) solutions. The uronic acid content, determined by the carbazole method, was $1.55 \pm 0.08$ per cent for mucin and $11.1 \pm 1.1$ per cent for mucoitinsulfuric acid. The reproducibility of the carbazole method therefore approximates that of our naphthoresorcinol method. In view of the greater simplicity of the carbazole method, and especially in view of its specificity, it deserves to be explored further with the purpose of applying it to the estimation of mucin in gastric secretion and the gastric contents.

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THE ESTIMATION OF MUCIN IN GASTRIC JUICE
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