Abstract:
Hydrochloric acid hydrolysed cellulose residues were treated with saliva amylase and the extent of this ptyelin action upon the insoluble cellulose residue was determined by quantitatively measuring the amount of reducing sugars produced.

A study was made of the effects of acid concentration, duration of acid hydrolysis, and the temperature used during acid hydrolysis on the amount of cellulose residue which would be hydrolyzed by ptyalin.

Since normal cellulose is not susceptible to ptyalin hydrolysis, a theory has been proposed to explain the action of saliva amylase on the insoluble cellulose residues.
PTYALIN ACTION ON INSOLUBLE ACID-TREATED CELLULOSE RESIDUES

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CELLULOSE RESIDUE WAS DETERMINED BY QUANTITATIVELY MEASURING THE AMOUNT OF
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Since normal cellulose is not susceptible to ptyalin hydrolysis, a
theory has been proposed to explain the action of saliva amylase on the
insoluble cellulose residues.
II. INTRODUCTION

It is well known that the potentially rich carbohydrate source of cellulose as an energy food is denied most of the animal world. A few of the lower animals can utilize cellulose directly as a source of energy but the higher animals are compelled to utilize it, at least in the major part, in a roundabout way.

With this in mind, this problem was conceived in the hope that some simple procedure could be found whereby cellulose could, to some extent, be made more directly available to animal life. If a practical solution is not forthcoming in this work, it is hoped that it will arouse interest in and further work to solve the many problems visualized in the course of this study.
Until a number of years ago when harvesting of wheat by combines became common on small farms, threshing of this grain left many straw piles on Montana farms which were eventually eaten by livestock, burned or decayed. Though farm animals were known to subsist on these straw piles for long periods with little other supplementation, it was nevertheless recognized that straw was a poor food at best, especially for winter feeding.

Straw, however, is potentially richer in carbohydrates than one might suspect from these observations. The crude fiber fraction is high (40-50%) and is not utilized greatly by animals. This engendered the thought that possibly this fraction could be resolved into more nutritive materials by chemical treatment and thus enable these animals to survive the winters on ranges and farms in a much better condition. Experiments centering about dilute acid treatment of straw in stacks was instituted by B. L. Johnson of the Department of Chemistry at Montana State College in 1935, and the changes in the crude fiber fraction and the accompanying increases in the reducing sugars were studied.

The present study of acid-treated cellulose residues grew out of the above mentioned findings. The maximum decrease in crude fiber by prolonged 0.5 N hydrochloric acid hydrolysis was never more than 10 percent. The increase in reducing sugars was large on a percentage basis, only because the amount present before treatment was almost negligible. However, animal tests were not conducted, and, therefore, it was not determined whether the acid treatment offered a greater increase in nutritive value than indicated by the changes in the amount of crude fiber and reducing sugars.
sugars. Inasmuch as part of the crude fiber undoubtedly consists of cellulose as well as of lignin, and especially inasmuch as lignin is undoubtedly combined with cellulose, it was thought acid treated cellulose might possibly give rise to insoluble residues which would be susceptible to amylolytic cleavage to reducing sugars. Rough preliminary tests indicated that saliva amylase (ptyalin) did hydrolyze the insoluble acid treated cellulose residues to a small but measurable degree.

Scientific literature from all over the world is abundant in the work of investigators who have studied the partial hydrolysis of straw and other crude fibers and the utilization of the insoluble partially hydrolyzed residues as a foodstuff (2,3,5,6,8). Most of this work has, however, been carried out by means of alkali hydrolysis and very little, if any, studies are published on the use of acids. Many of the results obtained by using the residues as food materials are only superficial, and it appears that no attempt has been made to determine quantitatively how well they could be utilized by the animal. Therefore the following study is aimed at a determination of the factors controlling the amount of ptyalin hydrolyzable fraction of residues obtained in incomplete acid hydrolysis of cellulose.
IV. EXPERIMENTAL

1. Source of Cellulose

Hexagon brand laboratory filter paper manufactured by Schaar and Company was used as the source of cellulose for this work. A large enough supply of the same brand and shipment was obtained at the beginning of the research to last throughout the entire experiment. It was hoped in this way to eliminate as much as possible any variations in the source of cellulose due to differences in brands or variations in the same brand.

Preliminary tests upon the filter paper gave no indication of the presence of residual reducing sugars. Preliminary tests also showed that the filter paper was not acted upon by saliva amylase in such a way as to produce any reducing sugars.

2. Treatment of the Cellulose by Hydrochloric Acid Hydrolysis with Respect to Acid Concentration, Temperature, and Time

The treatment of the cellulose prior to amylase action was carried out with two major variations in procedure, that of acid concentration and temperature.

In one case, the filter paper was put in concentrated hydrochloric acid which was kept at room temperature. At predetermined but arbitrary times, sub-samples of the cellulose residue, of such a size as to give convenient quantities for later study, were removed from the acid. The excess liquor was drained from these samples and the residue put in the open air to dry. The approximate time from introduction into the concentrated acid to complete dryness was recorded for each sub-sample.
The dry residues were then ground and washed with hot water, first by
decanting the supernatant liquid after the residue had settled, and, finally,
by filtering with a gravity filter. It was found that gravity filtration
gave better results than suction filtration, and in each case the washing
of the residue was continued until the supernatant liquid gave a negative
test for reducing sugars and chloride ions.

The washed residue was again put in the open air to dry and, when
completely dry, pulverized as before. After this, the finely powdered
residue was preserved in labeled, well stoppered bottles for the final
tests to be discussed later.

It might be pertinent at this point to give a more detailed discussion
of the drying and grinding of the samples, since it was found that certain
steps facilitated later treatments.

It was found that occasional stirring of the residue not only shorten­
ed the time necessary for drying but in the case of the unwashed acid
residue it greatly increased the ease with which the residue could be han­
dled during the first grinding process. It was found that in many cases
this acid residue, when dry, formed an extremely hard mass which could be
handled by the grinder much more easily when in small chunks, formed by
occasional stirring during the drying process. Since the grinder used did
not give the residue the desired degree of fineness, pulverization was
continued in a large mortar until the desired fineness was achieved. Fre­
quart stirring of the washed residues during drying was not as essential
since in this case the residue did not form hard masses, and usually only
pulverization in the mortar was all that was necessary to obtain the de­
sired degree of fineness for the dry washed residue.

In the second procedure, the cellulose samples were refluxed with the hydrochloric acid for various periods of time. Under these conditions, it was found by previous tests that about $3 \text{ M HCl}$ was the highest concentration that could be used without extreme discoloration of the residue after long periods of refluxing. On this basis, $3 \text{ M HCl}$ was used in this procedure.

At recorded intervals of time, refluxing of the sample was stopped and the acid residue suspension was divided into two equal parts. Both parts were allowed to settle a few minutes and the supernatant liquid was decanted. One of the halves was then neutralized with ammonium hydroxide, while the other was left acid. Both parts were then filtered by gravity filtration and the residues dried, ground, washed, etc., as before. Washing on these samples was continued, however, until all soluble coloring matter was removed to a point where it would not affect later colorimeter tests.

This refluxing procedure was also carried out on a few samples using $0.5 \text{ M HCl}$ instead of $3 \text{ M HCl}$.

3. Saliva Treatment of the Hydrolysis Residue

It was found by previous trial runs that room temperature was sufficient to give good saliva amylase action, and that 24 hours was more than sufficient time to complete any possible hydrolysis of the residue by the amylase at this temperature. Tests also showed that regulation of the pH was not necessary for this work, and that the chloride ions in the saliva were adequate for the activation of the amylase. In all samples dilution was made in the amount of one part of saliva to five parts of water by
volume. Dilution of the saliva appeared to introduce no disadvantages for this work but had the advantage of decreasing the volume of saliva necessary. After collection, the saliva was filtered to remove any solid matter which might be present.

The method of preparing samples to be run and necessary controls is probably best described by the following model experiment.

**Flask 1**
0.5 - 1.5 g residue + 5 cc of saliva + 25 cc of $\text{H}_2\text{O} + \text{Toluene}

**Flask 2 (First Control)**
0.5 - 1.5 g residue + 30 cc of $\text{H}_2\text{O} + \text{Toluene}

**Flask 3 (Second Control)**
5 cc of saliva + 25 cc of $\text{H}_2\text{O} + \text{Toluene}

The presence of Toluene in each flask was to insure against any bacterial action. The flasks were then stoppered and allowed to stand about 24 hours at room temperature with several shakings. At the end of the time, the mixtures were filtered and the filtrate was preserved in well stoppered containers.

The filtrate from flask 1 contained reducing sugars produced by amylase action on the residue plus any residual reducing sugars in the residue plus reducing fractions which might be present in the saliva. The quantity of reducing substances from these last two sources can be determined in the filtrates from flasks 2 and 3 respectively.

**4. Method of Reducing Sugar Determination**

Except for minor variations, the method used for reducing sugar determinations on the filtrate is the same as that of Folin-Wu(1) and Benedict(1).
In the Folin-Wu blood sugar determination, the blood filtrate is made protein free to eliminate any reducing groups due to proteins, but for this work this precaution is not necessary since any reduction due to the proteins in the saliva are very small and would also be included in the second control mentioned above.

Briefly outlined, the theory of the Folin-Wu determination is as follows: the filtrate is heated with an alkaline copper solution, and the reducing sugars in the filtrate quantitatively reduce the cupric to the cuprous ion. The cuprous salt formed is then permitted to react with molybdatephosphate solution. The molybdate is partially reduced by the cuprous ion to lower reduction products of blue color, the intensity of which is a measure of the amount of copper reduced to the cuprous state and, therefore, the amount of sugar present.

Procedure: Two cc of the unknown and two cc of a standard glucose solution were transferred to separate Folin-Wu blood sugar tubes. Two cc of alkaline copper reagent were added to each tube, and the tubes were well shaken. The tubes were placed in boiling water for six to eight minutes after which they were removed and cooled in a cold water bath for three minutes. Two cc of phosphomolybdic acid reagent were added subsequently to each tube and the contents well mixed by shaking for one minute. The solutions were diluted with water to the 25 cc mark on the tubes, after which the stoppered tubes were inverted several times to insure complete mixing, and the contents were then immediately compared colorimetrically. For this work, it was found that two or three unknowns could be compared with each
standard in a short enough time lapse so that no error was introduced by color changes due to standing.

Reagents:

Alkaline copper solution. 40 g of pure anhydrous sodium carbonate were dissolved in about 400 cc of water and transferred to a 1 liter volumetric flask. 7.50 g of tartaric acid were added and when dissolved 4.5 g of crystalline copper sulfate (CuSO₄·5H₂O) also added. The contents were mixed and made up to the mark with water and stored in an amber bottle.

Phosphomolybdate acid reagent. To 35 g of molybdic acid and 5 g of sodium tungstate were added 200 cc of 10% sodium hydroxide and 200 cc of water. The mixture was boiled in a beaker for thirty minutes, keeping the volume approximately constant by the addition of water from time to time. The solution was then cooled to room temperature and transferred to a 500 cc volumetric flask. 125 cc of concentrated phosphoric acid (syrupy 85%) were added. The contents were well mixed, diluted to the mark with water and stored in an amber bottle.

Standard glucose solution. The standard glucose solution was prepared from a stock glucose solution of convenient concentration. A saturated benzoic acid solution was used as the solvent in all cases to prevent any bacterial action, and all glucose solutions were stored either in amber bottles or kept in the dark. All standard solutions were compared against a standard glucose solution from the Bureau of Standards to determine their concentration. For this work, standard concentrations from 0.020-0.030 mg glucose/cc gave the best results.
5. Sample Calculations

Calculations were made with the washed acid-hydrolyzed residue on a near-as-possible dry weight basis. This was achieved by drying weighed samples of the residue in a vacuum oven at about 73°C for about 12 hours. The loss in weight was a measure of the water content of the residue and from this the percentage of non-volatile matter in the residue was calculated. Samples of the residue to be hydrolyzed with amylase were not dried with heat, in order to avoid any possible changes in the structure of the residue due to the elevated temperature and extreme drying.

A common Klett visual colorimeter was used to make all quantitative reducing sugar determinations. With this type of colorimeter, the following equation is employed in the calculation of mg of sugar.

\[
\frac{R_s}{R_u} \times \frac{C_s \times V}{v} \times \frac{D_u}{D_s} = C_u
\]

Where:
- \( R_s \) is the reading for the standard solution.
- \( R_u \) is the reading for the solution under test.
- \( C_s \) is the number of mg of sugar in the standard solution.
- \( V \) is the total volume of the unknown.
- \( v \) is the volume of unknown used in making the colorimeter test.
- \( D_u \) is the volume to which the unknown is diluted.
- \( D_s \) is the volume to which the standard is diluted.
- \( C_u \) is the mg of sugar per total volume of unknown.

The following model represents the calculations involved for a typical determination.
Unknown: 992.2 mg of residue (90.6% non-volatile) + 25 cc H₂O + 5 cc saliva

1st Control: 1019.8 mg of residue (90.6% non-volatile) + 30 cc H₂O

2nd Control: 25 cc H₂O + 5 cc saliva

The total concentration of glucose in the standard solution used for the colorimeter test is 0.022 mg/cc times the 2 cc used which gives 0.044 mg.

The colorimeter readings were as follows:

- Standard solution: 15.0
- Unknown: 5.5
- 1st control: 11.8
- 2nd control: 29.0

Calculation of the total mg of sugar (C) in the "unknown" above using the equation given above would be as follows:

\[
\frac{15}{5.5} \times 0.044 \times \frac{30}{2} \times \frac{25}{25} = 1.80 \text{ mg} \tag{A}^{1}
\]

Because of the necessity of controls, the following procedure must be followed in obtaining the final answer.

By calculating the "1st control" and "2nd control" by the same equation as above we obtain:

- total for the "1st control" is 0.64 mg (B) which is the reduction due to residual reducing substances in the residue.

- Total for the "2nd control" is 0.34 mg (C) which is the reduction due to reducing substances in the saliva.

---

1 All such values are labeled in this way for convenience.
By subtracting \((C)\) from \((A)\) we obtain:

\[
1.80 - .34 = 1.46 \text{ mg} \tag{D}
\]

which is the total mg of sugar in the "unknown" due only to amylase action and residual reducing substances in the residue.

By putting the residue in the "unknown" and "1st control" on a dry weight basis, we obtain:

for the "unknown": \[992.2 \times .906 = 898.9 \text{ mg} \tag{E}\]

for the "1st control": \[1019.8 \times .906 = 923.9 \text{ mg} \tag{F}\]

Dividing \((D)\) by \((E)\) gives the mg of sugar per mg of residue due only to amylase action and residual reducing substances in the residue.

\[
\frac{1.46}{898.9} = 1.625 \times 10^{-3} \text{ mg/mg} \tag{G}
\]

Dividing \((E)\) by \((F)\) gives the mg of sugar per mg of residue due to residual reducing substances in the residue.

\[
\frac{.84}{923.9} = .909 \times 10^{-3} \text{ mg/mg} \tag{H}
\]

By subtracting \((H)\) from \((G)\) we obtain the mg of reducing sugar per mg of residue actually due to amylase action.

\[
1.625 \times 10^{-3} - .909 \times 10^{-3} = .716 \times 10^{-3} \text{ mg/mg} \tag{I}
\]

Multiplying \((I)\) by \((E)\) gives the total mg of sugar in the "unknown" actually due to amylase action.

\[
.716 \times 10^{-3} \times 898.9 = .644 \text{ mg} \tag{J}
\]

or 0.072\% conversion, on a dry weight basis, of the HCl acid hydrolyzed residue to reducing sugars by amylase action.
V. RESULTS AND CONCLUSIONS

I. Results of Concentrated Hydrochloric Acid Hydrolysis for Various Lengths of Time at Room Temperature.

The residues for Tables I through VII were prepared by concentrated hydrochloric acid hydrolysis at room temperature. The time the cellulose spent in the acid solution and the time required to dry the acid residue heads each table. The longer the cellulose was in contact with the acid solution, the more difficult it became to air dry the large samples due to their changing consistency. The residue used in Tables IV and V is the same residue. They vary only by the time lapse between them, which is about three months. Table IV was the first test made, of course, and the rather interesting results which they show will be more thoroughly taken up later in the discussion and conclusions.

In all the following tables where they are used, all unknowns and controls were prepared according to the following standard procedure and nomenclature. Since these steps for each table are just a repetition of those before, it is not deemed necessary to repeat this introductory part for each set of tables.

"Unknown" (1 and 2) - residue + 25 cc H₂O + 5 cc saliva
"1st control" (3 and 4) - residue + 30 cc H₂O
"2nd control" (5 and 6) - 25 cc H₂O + 5 cc saliva

The total amount of sugar used in the standard was 0.044 mg.
### TABLE I. Time in Conc. Acid Solution - 3 Hours. Drying Time - 3 Days

<table>
<thead>
<tr>
<th>No. Wt. Residue</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar by %</th>
<th>Sugar by Amylase Conv. in mg</th>
<th>% Glucose by mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>916.2</td>
<td>95.9</td>
<td>878.6</td>
<td>10.0</td>
<td>27.4</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>960.8</td>
<td>95.9</td>
<td>921.4</td>
<td>10.0</td>
<td>28.5</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>822.6</td>
<td>95.9</td>
<td>788.9</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>831.6</td>
<td>95.9</td>
<td>797.5</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td>15.6</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>10.0</td>
<td>20.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### TABLE II. Time in Conc. Acid Solution - 18 Hours. Drying Time - 5 Days

<table>
<thead>
<tr>
<th>No. Wt. Residue</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar by %</th>
<th>Sugar by Amylase Conv. in mg</th>
<th>% Glucose by mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>786.9</td>
<td>95.2</td>
<td>749.1</td>
<td>15.0</td>
<td>12.0</td>
<td>0.83</td>
<td>Negative results, therefore not significant</td>
</tr>
<tr>
<td>2</td>
<td>995.9</td>
<td>95.2</td>
<td>948.1</td>
<td>15.0</td>
<td>14.0</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1043.2</td>
<td>95.2</td>
<td>993.1</td>
<td>15.0</td>
<td>19.0</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1042.3</td>
<td>95.2</td>
<td>992.3</td>
<td>15.0</td>
<td>26.2</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
<td>16.5</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>15.0</td>
<td>20.0</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Negative results, therefore not significant.*
### TABLE III. Time in Conc. Acid Solution - 22 Hours. Drying Time - 6 Days

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue (mg)</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue (mg)</th>
<th>Standard Color Setting</th>
<th>Color Reading</th>
<th>Total Sugar by Con- in mg</th>
<th>Amylase Con- in mg</th>
<th>% Glucose Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1175.7</td>
<td>95.7</td>
<td>1125.1</td>
<td>15.0</td>
<td>11.0</td>
<td>.90</td>
<td>.26</td>
<td>.023</td>
</tr>
<tr>
<td>2</td>
<td>1154.6</td>
<td>95.7</td>
<td>1105.0</td>
<td>15.0</td>
<td>14.2</td>
<td>.70</td>
<td>.07</td>
<td>.01</td>
</tr>
<tr>
<td>3</td>
<td>1101.6</td>
<td>95.7</td>
<td>1054.2</td>
<td>15.0</td>
<td>31.5</td>
<td>.31</td>
<td>.25 x 10^-3 ave. mg sugar/mg res. not due to amylase action</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1171.8</td>
<td>95.7</td>
<td>1121.4</td>
<td>15.0</td>
<td>39.0</td>
<td>.25</td>
<td>.37 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE IV. Time in Conc. Acid Solution - 42 Hours. Drying Time - 12 Days

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue (mg)</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue (mg)</th>
<th>Standard Color Setting</th>
<th>Color Reading</th>
<th>Total Sugar by Con- in mg</th>
<th>Amylase Con- in mg</th>
<th>% Glucose Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>992.2</td>
<td>90.6</td>
<td>898.9</td>
<td>15.0</td>
<td>5.5</td>
<td>1.80</td>
<td>.60</td>
<td>.067</td>
</tr>
<tr>
<td>2</td>
<td>1240.4</td>
<td>90.6</td>
<td>1123.8</td>
<td>15.0</td>
<td>4.5</td>
<td>2.20</td>
<td>.28</td>
<td>.069</td>
</tr>
<tr>
<td>3</td>
<td>1019.8</td>
<td>90.6</td>
<td>923.9</td>
<td>15.0</td>
<td>11.8</td>
<td>.84</td>
<td>.96 x 10^-3 ave. mg sugar/mg res. not due to amylase action</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1130.1</td>
<td>90.6</td>
<td>1023.9</td>
<td>15.0</td>
<td>9.5</td>
<td>1.04</td>
<td>.34 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
### TABLE V. Time in Conc. Acid Solution - 42 Hours. Drying Time - 12 Days

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Color Reading</th>
<th>Total Sugar by %</th>
<th>Sugar Amylase in mg</th>
<th>Conv. in version</th>
<th>Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>919.5</td>
<td>96.4</td>
<td>886.4</td>
<td>20.0</td>
<td>5.5</td>
<td>2.40</td>
<td>0.04</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>955.3</td>
<td>96.4</td>
<td>920.9</td>
<td>20.0</td>
<td>5.0</td>
<td>2.64</td>
<td>0.20</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1223.9</td>
<td>96.4</td>
<td>1179.8</td>
<td>20.0</td>
<td>5.5</td>
<td>2.40</td>
<td>2.11 x 10^{-3} ave. mg sugar/mg res. not due to amylase action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1124.1</td>
<td>96.4</td>
<td>1083.6</td>
<td>20.0</td>
<td>5.5</td>
<td>2.40</td>
<td>0.49 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>13.0</td>
<td>10.0</td>
<td>20.0</td>
<td>4.7</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>14.2</td>
<td>10.0</td>
<td>20.0</td>
<td>4.7</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE VI. Time in Conc. Acid Solution - 48 Hours. Drying Time - 15 Days

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Color Reading</th>
<th>Total Sugar by %</th>
<th>Sugar Amylase in mg</th>
<th>Conv. in version</th>
<th>Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1002.3</td>
<td>96.2</td>
<td>964.2</td>
<td>15.0</td>
<td>16.5</td>
<td>0.60</td>
<td>0.09</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>997.9</td>
<td>96.2</td>
<td>960.0</td>
<td>15.0</td>
<td>19.5</td>
<td>0.51</td>
<td>0.209 x 10^{-3} ave. mg sugar/mg res. not due to amylase action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1026.4</td>
<td>96.2</td>
<td>987.4</td>
<td>5.0</td>
<td>23.5</td>
<td>0.14</td>
<td>0.31 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1092.7</td>
<td>96.2</td>
<td>1051.2</td>
<td>15.0</td>
<td>34.5</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>33.2</td>
<td>15.0</td>
<td>32.5</td>
<td>30.0</td>
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<tr>
<td>6</td>
<td>15.0</td>
<td>32.5</td>
<td>15.0</td>
<td>32.5</td>
<td>31.0</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Wt. Residue mg</td>
<td>% Non-Volatile Residue</td>
<td>Dry Wt. Residue mg</td>
<td>Standard Color, Setting</td>
<td>Color Reading mg</td>
<td>Total Sugar by Glucose mg</td>
<td>Sugar by Amylase Conv. in mg</td>
<td>% Amylase Conversion</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>----------------</td>
<td>------------------------</td>
<td>--------------------</td>
<td>------------------------</td>
<td>----------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
<td>----------------------</td>
<td></td>
</tr>
<tr>
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<td>1018.5</td>
<td>96.3</td>
<td>980.8</td>
<td>15.0</td>
<td>15.5</td>
<td>.64</td>
<td>negative results, therefore not significant.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1028.1</td>
<td>96.3</td>
<td>990.1</td>
<td>15.0</td>
<td>13.5</td>
<td>.73</td>
<td>.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1091.8</td>
<td>96.3</td>
<td>1051.4</td>
<td>15.0</td>
<td>26.0</td>
<td>.38</td>
<td>.35 x 10^{-3} ave. mg sugar/mg res. not due: to amylase action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1022.9</td>
<td>96.3</td>
<td>985.1</td>
<td>15.0</td>
<td>29.0</td>
<td>.34</td>
<td>.35 mg ave. total sugar 30 cc sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>due to saliva sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For clarification and to facilitate comparison of the results in Tables I through VII, the percentage conversions for each table have been compiled in Table VIII below.

### TABLE VIII. Compiled Results of Tables I through VII

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Time in Acid Solution (Hrs.)</th>
<th>Drying Time (Days)</th>
<th>Unknown</th>
<th>% Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.025</td>
</tr>
<tr>
<td>II</td>
<td>18</td>
<td>5</td>
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<td>negative value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>negative value</td>
</tr>
<tr>
<td>III</td>
<td>22</td>
<td>8</td>
<td>1</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>IV</td>
<td>42</td>
<td>12</td>
<td>1</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.069</td>
</tr>
<tr>
<td>V</td>
<td>42</td>
<td>12</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.002</td>
</tr>
<tr>
<td>VI</td>
<td>48</td>
<td>15</td>
<td>1</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>negative value</td>
</tr>
<tr>
<td>VII</td>
<td>66</td>
<td>20</td>
<td>1</td>
<td>negative value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**Discussion:**

Rarely during the course of these and later determinations was it possible to obtain relatively close results for duplicate samples. Therefore, only trends in results could be compared with any satisfaction. Possibly more experimental study and refinement of technique could eliminate these discrepancies, but the accomplishment of this was beyond the time allotted for this paper.
From Table VIII it can be seen that 42 hours (Table IV) of acid hydrolysis under these conditions appears to have given a residue with the highest percentage of conversion to reducing sugars by ptyalin action. Above and below this time of acid hydrolysis (with the exception of 18 hours where the value was zero and Table V which is on the same residue as in IV as explained above), there is a general decrease in percentage conversion with decreasing and increasing times of acid hydrolysis.

At the time of preparation of these residues, each was washed until it gave both a negative Folin-Wu reducing sugar and chloride test. However, when determinations were run on these residues a month to 45 days later, each, with the exception of the 3 hour residue, contained residual reducing sugars, which becomes evident in the "1st control" (see tables).

A closer inspection of Tables IV and V, which, as mentioned above, are determinations on the same residue, with IV first, and V about three months later, reveals some peculiarities. The first determination gave a much higher conversion to reducing sugars than did the second. There was also a marked increase in the residual reducing sugars in the "1st control" which had increased from 0.096% for the first determination to about 0.21% for the second. Along with these changes, there was a decrease in the moisture content of the residue (about 9.4% moisture for the first and about 3.6% moisture for the second determination), even though the residue was kept in a tightly closed container, as were all the residues.

If further work should prove the validity of these apparent changes, this might prove to be an interesting problem for further study.
2. Results of 3 N Hydrochloric Acid Hydrolysis for Various Lengths of Time at Boiling Temperature

(A) Ammonium Hydroxide neutralized portion

The residues for Tables IX through XIII were prepared by 3 N hydrochloric acid hydrolysis at boiling temperature and the time of hydrolysis in each case heads each table. As mentioned earlier, the residues from the 3 N hydrochloric acid hydrolysis were divided into two equal parts, one of which was neutralized with NH₄OH solution before the first drying while the other half was left to dry in the acid state. The residues in the following Tables IX through XIII were neutralized before the first drying.

All unknowns and controls were prepared according to the standard procedure and nomenclature as outlined previously.
TABLE IX. Duration of 3 N Acid Hydrolysis - 1 Hour

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Color. Reading</th>
<th>Total Sugar by Glucose mg Glucose</th>
<th>Sugar by Amylase Con- in mg conv. in version</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>679.2</td>
<td>98.0</td>
<td>665.6</td>
<td>15.0</td>
<td>26.0</td>
<td>.38</td>
<td>negative results, therefore, not significant</td>
</tr>
<tr>
<td>2</td>
<td>835.2</td>
<td>98.0</td>
<td>818.5</td>
<td>15.0</td>
<td>20.0</td>
<td>.50</td>
<td>0.00 ave. mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>3</td>
<td>1017.0</td>
<td>98.0</td>
<td>996.7</td>
<td>15.0</td>
<td>0.00</td>
<td>.51 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>712.6</td>
<td>98.0</td>
<td>698.4</td>
<td>15.0</td>
<td>0.00</td>
<td>.52</td>
<td>0.00 ave. mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE X. Duration of 3 N Acid Hydrolysis - 2 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Color. Reading</th>
<th>Total Sugar by Glucose mg Glucose</th>
<th>Sugar by Amylase Con- in mg conv. in version</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>797.8</td>
<td>97.4</td>
<td>777.1</td>
<td>10.0</td>
<td>23.0</td>
<td>.29</td>
<td>.01 .001</td>
</tr>
<tr>
<td>2</td>
<td>1055.0</td>
<td>97.4</td>
<td>1027.6</td>
<td>10.0</td>
<td>21.5</td>
<td>.31</td>
<td>.03 .003</td>
</tr>
<tr>
<td>3</td>
<td>1144.1</td>
<td>97.4</td>
<td>1144.4</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00 ave. mg sugar/mg res. not due to amylase action</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1165.6</td>
<td>97.4</td>
<td>1135.3</td>
<td>10.0</td>
<td>0.00</td>
<td>0.28 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
### TABLE XI. Duration of 3 N Acid Hydrolysis - 3 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue mg</th>
<th>Standard Color Setting</th>
<th>Color. Reading</th>
<th>Total Sugar in mg Glucose</th>
<th>Sugar by Amylase in mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>811.4</td>
<td>97.9</td>
<td>794.4</td>
<td>10.0</td>
<td>30.0</td>
<td>.22</td>
<td>negative results, therefore, not significant</td>
</tr>
<tr>
<td>2</td>
<td>806.6</td>
<td>97.9</td>
<td>789.7</td>
<td>10.0</td>
<td>29.0</td>
<td>.23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>968.0</td>
<td>97.9</td>
<td>947.7</td>
<td>10.0</td>
<td>0.00</td>
<td>.24</td>
<td>0.00 mg ave. sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>4</td>
<td>950.4</td>
<td>97.9</td>
<td>930.4</td>
<td>10.0</td>
<td>0.00</td>
<td>.24</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>23 mg ave. total sugar/30 cc sample due to saliva sugar</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE XII. Duration of 3 N Acid Hydrolysis - 6 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue mg</th>
<th>Standard Color Setting</th>
<th>Color. Reading</th>
<th>Total Sugar in mg Glucose</th>
<th>Sugar by Amylase in mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1165.2</td>
<td>97.6</td>
<td>1137.2</td>
<td>10.0</td>
<td>22.0</td>
<td>.30</td>
<td>.10</td>
</tr>
<tr>
<td>2</td>
<td>1093.6</td>
<td>97.6</td>
<td>1067.4</td>
<td>10.0</td>
<td>31.0</td>
<td>.21</td>
<td>.01</td>
</tr>
<tr>
<td>3</td>
<td>1357.0</td>
<td>97.6</td>
<td>1324.4</td>
<td>10.0</td>
<td>0.00</td>
<td>.21</td>
<td>0.00 ave. mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>4</td>
<td>1188.2</td>
<td>97.6</td>
<td>1159.7</td>
<td>10.0</td>
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<td>.21</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>.20 mg ave. total sugar/30 cc sample due to saliva sugar</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table XIII. Duration of 3 N Acid Hydrolysis - 15 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt.</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar in mg</th>
<th>Sugar Amylase Conversion %</th>
<th>Residue not due to amylase action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1096.4</td>
<td>97.8</td>
<td>1072.3</td>
<td>10.0</td>
<td>25.0</td>
<td>.26</td>
<td>.07</td>
<td>.007</td>
</tr>
<tr>
<td>2</td>
<td>1143.0</td>
<td>97.8</td>
<td>1117.8</td>
<td>10.0</td>
<td>26.0</td>
<td>.25</td>
<td>.06</td>
<td>.005</td>
</tr>
<tr>
<td>3</td>
<td>1130.2</td>
<td>97.8</td>
<td>1105.3</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00 ave. mg sugar/mg glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1325.8</td>
<td>97.8</td>
<td>1296.6</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00 ave. mg sugar/mg glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>34.5</td>
<td>.19</td>
<td>0.19 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>35.0</td>
<td>.19</td>
<td>0.19 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
For clarification and to facilitate comparison of the results in Tables IX through XIII, the percentage conversion for each table has been compiled in Table XIV below.

**TABLE XIV. Compiled Results of Tables IX through XIII**

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Time in Acid Solution-Hrs.</th>
<th>Unknown</th>
<th>% Conversion</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>values</td>
</tr>
<tr>
<td>X</td>
<td>2</td>
<td>1</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>.003</td>
</tr>
<tr>
<td>XI</td>
<td>3</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>values</td>
</tr>
<tr>
<td>XII</td>
<td>6</td>
<td>1</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>.001</td>
</tr>
<tr>
<td>XIII</td>
<td>15</td>
<td>1</td>
<td>.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>.005</td>
</tr>
</tbody>
</table>

**Discussion:**

As can be seen by studying Tables IX through XIII and the compiled results in Table XIV above, this particular treatment of cellulose gave very low results and the establishment of any trends from the different times of hydrolysis is almost useless. It is evident, however, that this treatment gave much lower and less uniform results than the treatment with concentrated hydrochloric acid in the first set of tables.

None of these residues contained any residual reducing sugars, as can be seen by referring to the "1st control" in each table. This might possibly be explained by the fact that these residues had a much shorter time lapse between preparation and actual testing than did those residues in Tables I through VII. Most of these residues were tested within a week.
after their preparation.

(B) Portions dried in the acid state

The residues in the following tables, XV through XIX, were prepared at the same time as those under (A) and differed from them only in that these completed the first drying in the acid state. This drying process required about 36 hours, after which they were washed to negative reducing sugar and chloride tests and then prepared and tested by the standard procedure.
### TABLE XV. Duration of 3 N Acid Hydrolysis - 1 Hour

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue mg</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar in mg</th>
<th>Sugar Amylase Con-</th>
<th>% Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1024.0</td>
<td>97.2</td>
<td>995.3</td>
<td>15.0</td>
<td>15.0</td>
<td>.66</td>
<td>.15</td>
<td>.015</td>
</tr>
<tr>
<td>2</td>
<td>993.4</td>
<td>97.2</td>
<td>965.6</td>
<td>15.0</td>
<td>17.5</td>
<td>.57</td>
<td>.06</td>
<td>.006</td>
</tr>
<tr>
<td>3</td>
<td>1020.4</td>
<td>97.2</td>
<td>991.8</td>
<td>15.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>4</td>
<td>1024.6</td>
<td>97.2</td>
<td>995.9</td>
<td>15.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>0.00</td>
<td>15.0</td>
<td>19.0</td>
<td>.52</td>
<td>.51 mg ave. total sugar/30 cc sample due to saliva action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>15.0</td>
<td>20.0</td>
<td>15.0</td>
<td>20.0</td>
<td>.50</td>
<td>.50 mg ave. total sugar/30 cc sample due to saliva action</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE XVI. Duration of 3 N Acid Hydrolysis - 2 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue mg</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar in mg</th>
<th>Sugar Amylase Con-</th>
<th>% Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1451.3</td>
<td>97.4</td>
<td>1412.6</td>
<td>10.0</td>
<td>17.0</td>
<td>.39</td>
<td>negative results, therefore, not significant</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1435.4</td>
<td>97.4</td>
<td>1398.1</td>
<td>10.0</td>
<td>16.5</td>
<td>.40</td>
<td>.151 x 10⁻³ ave. mg sugar/mg res. not due to saliva action</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1326.7</td>
<td>97.4</td>
<td>1292.2</td>
<td>10.0</td>
<td>34.5</td>
<td>.19</td>
<td>.28 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1259.2</td>
<td>97.4</td>
<td>1226.5</td>
<td>10.0</td>
<td>35.0</td>
<td>.19</td>
<td>.28 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>22.0</td>
<td>10.0</td>
<td>25.0</td>
<td>.26</td>
<td>.26 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE XVII. Duration of 3 N Acid Hydrolysis - 3 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Total Sugar Residue</th>
<th>Amylase Reading</th>
<th>Amylase Conversion in mg Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>918.2</td>
<td>97.7</td>
<td>897.1</td>
<td>10.0</td>
<td>19.5</td>
<td>.34</td>
<td>.11</td>
</tr>
<tr>
<td>2</td>
<td>913.6</td>
<td>97.7</td>
<td>892.6</td>
<td>10.0</td>
<td>21.0</td>
<td>.31</td>
<td>.08</td>
</tr>
<tr>
<td>3</td>
<td>990.6</td>
<td>97.7</td>
<td>967.8</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sucrose/mg res. not due to amylase:</td>
</tr>
<tr>
<td>4</td>
<td>1137.6</td>
<td>97.7</td>
<td>1111.4</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sucrose/mg res. not due to amylase:</td>
</tr>
<tr>
<td>5</td>
<td>1137.6</td>
<td>97.7</td>
<td>1111.4</td>
<td>10.0</td>
<td>27.0</td>
<td>.24</td>
<td>0.23 mg ave. total sugar/30 cc sample due</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE XVIII. Duration of 3 N Acid Hydrolysis - 6 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt. Residue</th>
<th>Standard Color Setting</th>
<th>Total Sugar Residue</th>
<th>Amylase Reading</th>
<th>Amylase Conversion in mg Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1118.8</td>
<td>97.8</td>
<td>1094.2</td>
<td>10.0</td>
<td>16.0</td>
<td>.41</td>
<td>.21</td>
</tr>
<tr>
<td>2</td>
<td>1157.1</td>
<td>97.8</td>
<td>1131.6</td>
<td>10.0</td>
<td>14.5</td>
<td>.46</td>
<td>.26</td>
</tr>
<tr>
<td>3</td>
<td>1106.8</td>
<td>97.8</td>
<td>1082.4</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sucrose/mg res. not due to amylase:</td>
</tr>
<tr>
<td>4</td>
<td>1156.4</td>
<td>97.8</td>
<td>1131.0</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00 mg sucrose/mg res. not due to amylase:</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>32.0</td>
<td></td>
<td></td>
<td>32.0</td>
<td>.21</td>
<td>0.20 mg ave. total sugar/30 cc sample due</td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>35.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE XIX. Duration of 3 N Acid Hydrolysis - 15 Hours

<table>
<thead>
<tr>
<th>No.</th>
<th>Wt. Residue mg</th>
<th>% Non-Volatile Residue</th>
<th>Dry Wt.</th>
<th>Standard Color. Setting</th>
<th>Color. Reading</th>
<th>Total Sugar by % Sugar Amylase in mg conv. in Glucose mg Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1134.2</td>
<td>98.2</td>
<td>1113.8</td>
<td>10.0</td>
<td>19.0</td>
<td>.35</td>
</tr>
<tr>
<td>2</td>
<td>1134.5</td>
<td>98.2</td>
<td>1114.1</td>
<td>10.0</td>
<td>21.0</td>
<td>.31</td>
</tr>
<tr>
<td>3</td>
<td>1325.3</td>
<td>98.2</td>
<td>1301.4</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00 ave. mg sugar/mg res. not due to amylase action</td>
</tr>
<tr>
<td>4</td>
<td>1146.4</td>
<td>98.2</td>
<td>1125.8</td>
<td>10.0</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>34.5</td>
<td>.19</td>
<td>.19 mg ave. total sugar/30 cc sample due to saliva sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10.0</td>
<td>35.0</td>
<td>.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
As before, the results of Tables XV through XIX have been compiled in Table XX below.

### TABLE XX. Compiled Results of Tables XV through XIX

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Time in Acid Solution-Hrs.</th>
<th>Unknown</th>
<th>% Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>XV</td>
<td>1</td>
<td>1</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.006</td>
</tr>
<tr>
<td>XVI</td>
<td>2</td>
<td>1</td>
<td>negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>values</td>
</tr>
<tr>
<td>XVII</td>
<td>3</td>
<td>1</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.009</td>
</tr>
<tr>
<td>XVIII</td>
<td>6</td>
<td>1</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>XIX</td>
<td>15</td>
<td>1</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.011</td>
</tr>
</tbody>
</table>

**Discussion:**

Drying in the acid state greatly increased the yield from these residues as compared to the same duration of hydrolysis in Tables IX through XIII. It is apparent, therefore, that the residues were still hydrolyzed by the concentrated hydrochloric acid which resulted during the drying. The residue in Table XVIII then gave the better yield.

The absence of residual reducing sugars, with the exception of Table XVI, can probably be explained again by the same reasoning that was given in Part (A).

### 3. Results of 0.5 M Hydrochloric Acid Hydrolysis at Boiling Temperature

The results from the treatment of cellulose with 0.5 M hydrochloric acid were not significantly different from those obtained on residues from the 3 M hydrochloric acid hydrolysis; therefore, it will not be necessary
to enter tables showing these results. It should suffice to state that the concentration of the acid at these low concentrations apparently had little effect upon the final results, even when the hydrolysis was carried out at the elevated temperatures employed.

4. Conclusions

Before proceeding further with this discussion, it might be well to state again the major purpose of this work: to study the effect of ptyalin hydrolysis of insoluble cellulose residues resulting from inorganic acid hydrolysis of cellulose. A study of the preceding tables would seem to indicate that ptyalin hydrolysis of the insoluble cellulose residues does take place, although its amount is rather varied throughout the different residues tested, and the total amount of hydrolysis was not as large or spectacular as it was hoped it might be. It is the belief of this author, however, that further study and modifications of techniques employed could greatly increase the desired obtainable results.

Pacsu and Hiller(7) have shown that the structure of cellulose is believed to be a lattice work of long beta-D-glucopyranose chains, which are held together by the hemiacetal linkages of glucose, cellobiose, cellotriose, etc. They go on to state that hydrolysis of cellulose by weak acids does not hydrolyze the 1,4-glucosidic bonds of the primary chain, but rather hydrolyzes hemiacetal bonds which connect the primary chain molecules together, thus giving rise to secondary chain molecules. This process, they state, results in a leaching-out of the smaller molecules such as glucose, cellobiose, etc., leaving a residue of large primary chains. According to Pacsu and Hiller, the hemiacetal bonds are readily broken by weak acids but
the 1,4-glucosidic bonds of the primary molecule chains are extremely resistant even to hydrolysis with highly concentrated acids and are, therefore, broken only very slowly.

If this theory of Faccu and Hiller is to be accepted, then the cellulose residues, known as hydrocellulose, which are used in this work, can only consist of primary molecular chains of the beta-1,4-glucosidic type.

It does not seem reasonable, however, to assume that all the bonds in these hydrocellulose chains are of the beta-linkage type as supposedly found in normal cellulose since it is well established that ptyalin is only active on linkages of the alpha-type as found in common starch. There is also little reason to doubt the alpha specificity of the enzyme.

All through this work the indications were that there was only a certain limited amount of the given residue which could be attacked by ptyalin, and length of time of amylase contact with the residue or treatment with fresh enzyme did not increase this given limited yield. This would further seem to eliminate any possibility of ptyalin action on beta-linkages.

It might be well at this point to include a statement by Stark(9) who is of the opinion that the products of the action of ptyalin on starch include glucose, maltose, and "an array of nonfermentable copper-reducing polysaccharides". Until further work has proven otherwise, it is not unreasonable to assume that the products of the action of ptyalin on hydrocellulose are the same as those which Stark believes are obtained by the action of ptyalin on starch. It is also believed that saliva amylase is composed of two fractions, that called beta-amylase or maltogenic amylase which attacks the long alpha-D-1,4-glucopyranose chains of starch, and
that part called alpha-amylase or dextrogenic amylase, which attacks the glucose, maltose, etc., cross linkages which hold together the long 1,4-glucosidic chains by alpha-hemiacetal linkages.

Keeping in mind the previous discussion, an attempt will now be made to derive a satisfactory explanation as to how and where ptyalin might attack hydrocellulose residues.

Even though Pacsu and Hiller have stated that 1,4-glycosidic linkages are broken only by strong acids, it is not beyond the realm of reason to assume that some of these linkages will be broken whether the acid is strong or weak, the greater hydrolysis, of course, being favored by the stronger acid. It should, therefore, not be out of order to assume that some of these broken linkages could reform, in part, as alpha-linkages. This would give an insoluble hydrocellulose molecule containing most of the original beta-glucosidic linkages and one or more alpha-glycosidic linkages which would be labile to ptyalin action.

The results obtained in this work appear to conform fairly well with this idea. The residues of Tables I through VII were prepared with concentrated acid which would favor greater hydrolysis of 1,4-glycosidic linkages and increase the possibilities of reformation of these linkages. On the other hand, the residues in Tables IX through XIII were hydrolyzed with a dilute acid, a condition not favorable to breaking of 1,4-glycosidic linkages, and this lower percentage of hydrolysis would give a corresponding lower percentage of reformation. The residues in Tables XV through XIX, while drying, were under conditions similar to those found in the prep-
aration of the residues in Tables I through VII. The increased ptyalin susceptible fraction over that of residues dried in the neutral state in Tables IX through XIII bears out the more favorable conditions of concentrated acid.

If the assumption that 1,4-glucosidic linkages can reform after they have been broken is valid, then it should also be valid to assume that they could reform as hemiacetal linkages instead of 1,4-glucosidic linkages. The reformation could give alpha-hemiacetals instead of beta-hemiacetals, and again we could have hydrocellulose molecules, part of which would be susceptible to ptyalin action. Hemiacetals are unstable and, as stated by Pecsu and Hiller, linkages of this type are readily broken by weak acids. It may be that small amounts of hydrochloric acid are bound to the hydrocellulose, to complete a loose oxonium structure, during acid hydrolysis and is not removed by washing which would leave the dry residue in a potentially acid state. This bound acid in the presence of the small percentage of moisture present in all the dried residues would favor the hydrolysis of the reformed hemiacetal linkages on long standing. These conditions might explain the appearance after a long time of the soluble sugars found in the well washed residues of Tables I through VII. This condition is particularly brought out in Tables IV and V, where an increase in soluble sugars in the residue was accompanied by a corresponding decrease in water content which was presumably used up, at least in part, in the hydrolysis of the hemiacetal bonds. Whether similar concepts have application in connection with starch is, at this time, entirely conjectural.
The time that the cellulose was in contact with the acid solution appears to be an important factor in all the sets of residues, since above and below a given time the percentage conversion to reducing sugars by ptyalin action decreases. The role that is played by time is, however, not well understood, therefore, it will probably have to await further study in this field for a satisfactory explanation of its influence.

In short the all-over reaction of acid hydrolysis of hydrocellulose appears to be an equilibrium between the hydrolysis of 1,4-glucosidic linkages and the reformation of these linkages as 1,4-glucosidic linkages and hemiacetal linkages.

Although the picture presented above might appear to be a fairly satisfactory explanation, the reader should be warned that all that can be concluded from this work is that acid hydrolysis of cellulose appears to give an insoluble residue which is partially labile to ptyalin action. Any attempt to explain the changes which have taken place in the residue during acid hydrolysis is still mostly theory and much more study along this line should be undertaken before these theories, if proven valid, can be accepted as facts.
VI. SUMMARY

1. Laboratory filter paper was used as the source of cellulose.

2. The cellulose was hydrolyzed for various lengths of time with both concentrated and dilute hydrochloric acid and at room temperature as well as boiling temperature. After this acid hydrolysis, the washed and dried cellulose residues were allowed to stand with dilute saliva solutions for about 24 hours, then filtered and the filtrate tested colorimetrically for reducing sugars.

3. The main objective of this work was fulfilled when it was found that in almost all cases the cellulose residue was apparently partially susceptible to ptyalin action. Since beta-linkages, as found in normal cellulose, are not attacked by ptyalin, this susceptibility of the residue was explained by assuming that in acid hydrolysis some of the 1,4-glucosidic linkages of the primary cellulose chains could be broken and then reform in an equilibrium reaction, as alpha linkages, thus making part of the residue labile to ptyalin action.

4. In certain instances, there were indications that the well washed and dried reducing-sugar-free residue, upon standing for extended periods in stoppered bottles, underwent further changes, since there were found present soluble reducing sugars, after this time, where there had been none originally. This was explained as possibly due to the reformation of broken 1,4-glucosidic linkages as hemiacetal linkages, which are easily hydrolyzed by weak acids. If these linkages were alpha-hemiacetals, they would also be susceptible to ptyalin action. It was then assumed that some hydrochloric acid was bound to the cellulose
residue, possibly to complete a loose oxonium structure. Liberated hydrogen ions from this bound acid plus the small amount of moisture which was present in all dry residues could hydrolyze these highly susceptible hemiacetal linkages, thus producing the reducing sugars which appeared in the residues after long periods of standing.

5. The length of time of acid hydrolysis apparently had an effect upon the amount of residue which would be hydrolyzed by ptyalin. How this was brought about was, however, not well understood. Therefore, no explanation of this phenomenon was attempted.
ACKNOWLEDGMENT

This author would like to take this opportunity to express his thanks and gratitude to Dr. B. L. Johnson, Professor of Chemistry at Montana State College, without whose kind assistance and guidance this work could not have been accomplished; to all those who in a more minor yet not less important way were instrumental in helping complete this paper; and last but certainly not least to my wife, who, though with only a meager knowledge of chemistry, was my severest critic and source of consolation whenever problems became difficult or overwhelming.
VIII. LITERATURE CITED AND CONSULTED

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(5) Hebst, W., C. A. 35:56987 (1941); (Fr. 847,587 (Oct. 12, 1939)).
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Ptyalin action on insoluble acid-treated cellulose residues

Sanborn, E. N.