Introduction

The endosperm of sweet corn, in contrast to that of most varieties of maize, contains a relatively high percentage of soluble polysaccharides, but very little is known concerning their physical and chemical properties. On the basis of the iodine color alone, Culpepper and Magoon (8) concluded that these water soluble polysaccharides are composed of both dextrins and soluble starch.

This investigation deals with the isolation and purification of the water soluble polysaccharides from green and mature Hopeland sweet corn kernels, and with their physical and chemical properties. These properties were compared with those of alpha and beta amylose from mature Hopeland sweet corn starch. The chief purpose of this comparison was to determine whether the soluble polysaccharides are true dextrins produced by starch hydrolysis or whether they are synthesized amyloses that have not been organized into starch grains.

Investigation

Extraction and Purification of Soluble Polysaccharides

The green sweet corn was prepared for extraction of the soluble polysaccharides by splitting the kernels and scraping out the contents. The air dry mature corn was first soaked in 20 per cent. alcohol and the softened kernels were then ground through a Nixtamal mill. Enzymatic action was checked during the extraction by $1 \times 10^{-4}$ N iodine (18).

These polysaccharides are soluble in water and in alcohol up to 30 per cent. by volume and are precipitated by alcohol above this concentration. Therefore 20 per cent. alcohol was used as the solvent during extraction and purification. Samples of the corn pulp were extracted twice with cold 20 per cent. alcohol. The first extraction was made soon after adding the alcohol while the second was made after shaking for one hour. The extracts were separated from the pulp by means of several thicknesses of cheesecloth. These extracts contained the soluble polysaccharides, starch, and various other substances. The starch was removed from the extracts by centrifuging and filtering. The soluble polysaccharides were then precipitated from the filtrate by increasing the alcoholic concentration to 60 per cent. by volume. The 60 per cent. alcohol was removed and the material was re-
dissolved in 20 per cent. alcohol. This solution was centrifuged and filtered in order to remove impurities. The process of dissolving in 20 per cent. alcohol and reprecipitating in 60 per cent. alcohol was repeated until no residue was deposited on centrifuging the 20 per cent. alcoholic solution and qualitative tests for proteins in the wash alcohol were negative. Final purification of the soluble polysaccharides was effected by dissolving in water, filtering, and subjecting the filtrate to electrodialysis. The apparatus (fig. 1) employed for electrodialysis is a modified form of the chamber used by

\[ \text{FIG. 1. Electrodialysis apparatus: } A, \text{ improved negative electrode chamber; } B, \text{ negative electrode chamber used until 1932; } C, \text{ siphon for dialysis chamber; } D, \text{ mercury connections for electrode; } E, \text{ siphon for electrode chamber; } F, \text{ dialysis chamber; } G, \text{ positive electrode chamber; } H, \text{ platinum electrodes; } I, \text{ collodion membranes.} \]

TAYLOR and IDDLES (23). The electrode chamber which was used for the purification of the samples taken in 1930 and 1931 is shown in figure 1B; the electrode chamber which was adopted in 1932 is shown in figure 1A. The new electrode chamber has a much larger membrane surface and allows
a larger volume of water to surround the electrode. This improvement hastened the separation and purification of the materials investigated. The membranes of the electrode chambers were of collodion supported on cheesecloth and were of such thickness that they allowed the free passage of inorganic ions while retaining the polysaccharides. A current of 250 volts d.c. was passed through the solutions until they were free of unbound inorganic ions. This point was detected by the conductivity of the water in the electrode chambers. The water in these chambers was changed frequently during the first 24 hours of a run; from then on once a day was sufficient.

The water soluble polysaccharides were divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions were tentatively named alpha and beta respectively. The purification of the alpha and beta fractions of the soluble polysaccharides was readily effected by siphoning the supernatant beta fraction from the chamber. Water was again added to the alpha fraction and electrodialysis was continued until a clear separation had occurred. This process was repeated until the water above the alpha fraction was free of the beta fraction, as evidenced by no precipitate in 60 per cent. alcohol and no coloration with iodine. The purified alpha and beta fractions were precipitated in 70 per cent. alcohol, washed in absolute alcohol, and dried in a vacuum over sulphuric acid at room temperature. Although the beta fraction always remained in suspension during electrodialysis, a portion of it migrated toward the positive electrode. The properties of this portion were identical with those of the non-migrating portion.

Separation and Purification of Alpha and Beta Amylose from Starch

The starch from air dry mature sweet corn which had been separated from the soluble polysaccharides by centrifuging was purified by washing in 20 and 80 per cent. alcohol. The starch was washed and centrifuged eleven times in 80 per cent. alcohol before it was considered pure.

It is well known that the starch grains must be completely ruptured before a separation of alpha and beta amylose can be made. In order to avoid the possibility of any change in the beta amylose arising from the use of chemical agents, the method of mechanical rupture as reported by Alsb erg and Perry (1) and Taylor and Beckmann (22) was adopted. This method gave complete rupture of the starch grains after grinding in a ball mill for one week, as evidenced by the disappearance of the typical black cross on the starch grains when viewed between crossed Nicol prisms.
An 8 per cent. suspension of ground starch in water was subjected to
electrodialysis. The alpha amylose migrated to the anode and the clear
supernatant beta amylose was siphoned off and precipitated in 75 per cent.
alcohol, washed in absolute alcohol, and dried in a vacuum at room tem-
perature. The alpha amylose was repeatedly resuspended in water and electro-
dialized until free of beta amylose. This required 59 washings. The alpha
amylose was then frozen and the frozen mass allowed to thaw as it was
filtered in a Buchner funnel. Drying was completed in a vacuum desiccator
over sulphuric acid at room temperature.

PHYSICAL AND CHEMICAL METHODS

COLOR WITH IODINE.—The iodine coloration of a solution was determined
by adding 10 drops of N/100 iodine potassium iodide to 10 ml. of a 1 per
cent. solution.

VISCOITY.—The relative viscosity was determined with an Ostwald visco-
simeter. The measurements were made on 5 ml. of a 1 per cent. solution
at 25° C. ± 0.03. All of the determinations were made immediately after
preparing the solutions. Each value for relative viscosity is the average
of at least two samples and each sample was run through the viscosimeter
ten times.

OPTICAL ROTATION.—A Schmidt and Haensch half-shadow saccharimeter
was used to determine the optical rotation. The readings were obtained in
Ventzke degrees which were converted to angular rotation by the factor
0.34657. It was necessary to use dilute solutions of the beta soluble poly-
saccharide preparations because of their opalescence. In determining the
optical rotation of samples which had been hydrolyzed, the original weight
of the sample was used to calculate the specific rotation. The specific rota-
tions reported for the unhydrolyzed samples are the average of two samples,
and five to six readings were made on each sample. The specific rotations
for the hydrolyzed materials are from one sample, on which five to six
readings were made.

ELECTRICAL CONDUCTIVITY.—The apparatus employed for these deter-
minations consisted of the usual equipment for such measurements and was
set up as shown by Getman (9). A Leeds and Northrup student’s poten-
tiometer was used as a wheatstone bridge. The determinations were made
on 1 per cent. solutions at 25° C. ± 0.03. Each conductivity reported is the
average of two samples. The conductivity of each sample was determined
nine or more times with the fixed resistance at three different points.

FLOCCULATION BY SALTS.—The samples of 10 ml. of 2 per cent. solutions
were treated with half saturated ammonium sulphate, basic lead acetate, and
0.1 N iodine potassium iodide.
Ash.—The materials were ashed in platinum crucibles at dull redness in a muffle furnace. The ashings were continued until the crucibles were constant in weight after 30-minute heating intervals.

Phosphorus.—All of the ashings for the phosphorus determinations were made by the wet ashing method following the procedure recommended by Zinzadze (25). The ashings were performed on an electric sand bath at 165°–170° C., since Roe, Irish, and Boyd (20) have shown that phosphoric acid is volatized above 200° C. The colorimetric method employed for the beta fractions is that of Bell and Doisy (5) as modified by Briggs (6) and improved by Roe, Irish, and Boyd (20). The procedure for this determination has been reported by Cockerfair (7). The standards were prepared from potassium dihydrogen phosphate simultaneously with the unknown solutions. Since Roe, Irish, and Boyd (20) have shown that the acidity has a marked influence on the color produced, it was necessary to add 1 ml. of concentrated sulphuric acid to the standards in order to make the acidity the same as that of the unknown solutions. The solutions were matched with a Bausch and Lomb Duboscq colorimeter, using 50 mm. cups.

The volumetric method employed for the alpha fractions is essentially that of Pemberton (19) as reported by Mathews (16). The ammonium phospho-molybdate was washed with water until free of acid, and then dissolved in N/10 sodium hydroxide. This solution was titrated with N/10 sulphuric acid.

Fatty Acids Before and After Hydrolysis.—The samples which had been dried at 80° C. under 3 to 4 cm. pressure were extracted in a Bailey-Walker (2) extraction apparatus for 24 hours with redistilled anhydrous ethyl ether. The extract was evaporated and dried at 80° under 3 to 4 cm. pressure. Any residue remaining after correcting for the blank determination was considered unbound or extraneous fatty material.

The extracted samples were hydrolyzed according to the method of Taylor and Nelson (24) and filtered through fat-free filter paper. The residues were washed free of acid, and after drying the filters and the residues at 50° C., they were extracted with anhydrous ethyl ether. The weight of the ether extract was determined in the manner previously described.

Reduction of Fehling’s Solution Before and After Hydrolysis.—The reducing power of 50 ml. of 1 per cent. solutions was determined by the Munson and Walker gravimetric procedure (17). The reducing power of samples which had been hydrolyzed for 2.5 hours with 2.5 per cent. sulphuric acid was also determined by this method. The percentage of glucose formed on hydrolysis was calculated by using the factor 0.9, which allows for the increase in weight on hydrolysis.

Melting Point of Osazones.—Samples which had been hydrolyzed were neutralized and treated with phenyl-hydrazine hydrochloride and sodium
acetate in a boiling water bath for 1 hour. The resulting osazones were recrystallized and their melting point determined by the capillary tube method. All of the melting points were corrected according to the formula of Kopp (11). The melting points reported are the averages of two or three determinations.

Nitrogen.—The nitrogen determinations were made by the usual Gunning method (17). Distillation was made into 0.1 N sulphuric acid and the excess acid was titrated with 0.1 N sodium hydroxide using the combination methylene blue-methyl red indicator (10).

Results

Properties of beta fraction of soluble polysaccharides prepared from sweet corn in different years

The beta fraction constitutes from 78 to 80 per cent. of the water soluble polysaccharides in sweet corn at the milk stage. The properties of this fraction of the soluble polysaccharides prepared from two different crops of sweet corn in the milk stage were practically identical. Both prepara-

TABLE I
Physical properties of beta fraction of soluble polysaccharides prepared from two crops of sweet corn in the milk stage

<table>
<thead>
<tr>
<th>Year</th>
<th>Relative Viscosity at 25° C. ± 0.03</th>
<th>Specific Conductivity at 25° C. ± 0.03 x 10^{-6}</th>
<th>Specific Rotation of Hydrolyzed Samples at 25° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1930</td>
<td>1.0715</td>
<td>9.40</td>
<td>195.8</td>
</tr>
<tr>
<td>1931</td>
<td>1.0750</td>
<td>3.39</td>
<td>195.2</td>
</tr>
</tbody>
</table>

TABLE II
Chemical properties of beta fraction of soluble polysaccharides prepared from two crops of sweet corn in the milk stage

<table>
<thead>
<tr>
<th>Year</th>
<th>P_{2}O_{5}</th>
<th>Conversion to Glucose on Hydrolysis</th>
<th>Melting Point of Osazones from Hydrolyzed Samples</th>
<th>Total Nitrogen*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1930</td>
<td>0.0079</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>1931</td>
<td>0.0013</td>
<td>95.22</td>
<td>208.8</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Single determination.
tions were white amorphous powders which were exceedingly hygroscopic. They were not flocculated by basic lead acetate, half saturated ammonium sulphate, or iodine potassium iodide. Neither of the beta preparations contained any weighable ash or any fatty material. Both preparations were non-reducing and exhibited the same purplish violet iodine color. The results of other physical and chemical measurements on these two preparations are shown in tables I and II. The material prepared in 1930 had a higher conductivity than the material prepared in 1931. This may be due to the fact that the 1930 preparation had more bound phosphorus and also more nitrogen. The nitrogen is probably due to impurities which had not been completely removed from the 1930 preparations.

**Comparison of Properties of Beta Fraction of Soluble Polysaccharides With Those of Beta Amylose Prepared From Mature Sweet Corn Starch**

Preparations of the beta fraction of the soluble polysaccharides from corn in three stages of ripening were compared with one another and with beta amylose from mature sweet corn starch. The solutions of beta fractions of soluble polysaccharides were very opalescent and the iodine color was purplish violet, while the beta amylose solutions were water-clear and gave a pure blue iodine color. There was no ash or bound fatty material in any of the beta fractions or in beta amylose. The materials compared did not reduce Fehling's solution and they were not flocculated by half-saturated ammonium sulphate, basic lead acetate, or iodine potassium iodide. On the addition of a small amount of an electrolyte to the solutions, the beta amylose was flocculated by iodine potassium iodide as reported by

**Table III**

**Comparison of Physical Properties of Beta Fraction of Soluble Polysaccharides and Beta Amylose From Sweet Corn Starch**

<table>
<thead>
<tr>
<th>Material</th>
<th>Relative Viscosity at 25°C ± 0.03</th>
<th>Specific Conductivity at 25°C ± 0.03 x 10^-6</th>
<th>Specific Rotation at 25°C</th>
<th>Specific Rotation of Hydrolyzed Samples at 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta fraction of soluble polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk stage</td>
<td>1.0750</td>
<td>3.39</td>
<td>195.2</td>
<td>58.7</td>
</tr>
<tr>
<td>Dough stage</td>
<td>1.0723</td>
<td>3.35</td>
<td>195.8</td>
<td>55.2</td>
</tr>
<tr>
<td>Mature stage</td>
<td>1.0850</td>
<td>6.40</td>
<td>195.8</td>
<td>54.5</td>
</tr>
<tr>
<td>Beta amylose</td>
<td>1.1690</td>
<td>15.10</td>
<td>214.0</td>
<td>60.6</td>
</tr>
</tbody>
</table>
Baldwin (4), but the beta fraction of the soluble polysaccharides was not affected by this treatment. The results of further studies of the physical and chemical properties of these preparations are tabulated in tables III and IV. The viscosities, conductivities, and specific rotations of the beta fraction of the soluble polysaccharides were not affected by this treatment.

The results of further studies of the physical and chemical properties of these preparations are tabulated in tables III and IV. The viscosities, conductivities, and specific rotations of the beta fraction of the soluble polysaccharides were not affected by this treatment.

### Table IV

**Comparison of Chemical Properties of Beta Fraction of Soluble Polysaccharides and Beta Amylose from Sweet Corn Starch**

<table>
<thead>
<tr>
<th>Material</th>
<th>$P_O_5$</th>
<th>Conversion to Glucose on Hydrolysis</th>
<th>Melting Point of Osazones from Hydrolyzed Samples</th>
<th>Total Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta fraction of soluble polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk stage</td>
<td>0.0013</td>
<td>97.96</td>
<td>205.9</td>
<td>0.01*</td>
</tr>
<tr>
<td>Dough stage</td>
<td>0.0000</td>
<td>98.37</td>
<td>204.6</td>
<td>0.07*</td>
</tr>
<tr>
<td>Mature stage</td>
<td>0.0045</td>
<td>94.50</td>
<td>210.6</td>
<td>0.05*</td>
</tr>
<tr>
<td>Beta amyllose</td>
<td>0.0042</td>
<td>92.88</td>
<td>209.4</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* Single determination.

The alpha fraction of the soluble polysaccharide preparations are less than those of beta amylose. The phosphorus content of the beta fraction from the mature stage and beta amylose is the same, and from the specific rotation of hydrolyzed samples and the melting point of osazones it is evident that the beta fraction of the soluble polysaccharides is converted to glucose on hydrolysis, as is beta amylose.

**Comparison of Properties of Alpha Fraction of Soluble Polysaccharides and Alpha Amylose**

The water soluble polysaccharides from the milk, dough, and mature stages contained approximately 22, 26, and 45 per cent. of the alpha fraction respectively, while the percentage of alpha amylose in the starch was approximately 14 per cent.

The properties of the alpha fraction and alpha amylose which have been compared are shown in tables V and VI. The iodine color of alpha amylose was violet while the iodine color of the alpha fraction was blue. As the corn matures the alpha fraction of the soluble polysaccharides loses in ash, phosphorus, and nitrogen; therefore the compound from mature corn had a higher percentage of conversion to glucose on hydrolysis. The optical rotation of the hydrolyzed samples varied with the stage of ripening of the corn.
PARKER: PROPERTIES OF POLYSACCHARIDES

TABLE V

COMPARISON OF ASH, PHOSPHORUS, AND NITROGEN CONTENT OF ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND ALPHA AMYLOSE FROM SWEET CORN STARCH

<table>
<thead>
<tr>
<th>Material</th>
<th>Ash %</th>
<th>P₂O₅ %</th>
<th>Total Nitrogen %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha fraction of soluble polysaccharides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk stage</td>
<td>0.113</td>
<td>0.674</td>
<td>3.33</td>
</tr>
<tr>
<td>Dough stage</td>
<td>0.080</td>
<td>0.074</td>
<td>0.94</td>
</tr>
<tr>
<td>Mature stage</td>
<td>0.017</td>
<td>0.025</td>
<td>0.19</td>
</tr>
<tr>
<td>Alpha amylose</td>
<td>0.800</td>
<td>0.164</td>
<td>0.26</td>
</tr>
</tbody>
</table>

corn from which they were prepared. However, if the weights of hydrolyzed samples were corrected for their percentage of conversion to glucose, the optical rotations approximated that of d-glucose, with the exception of the material from the milk stage, which was lower. The alpha fraction of the soluble polysaccharides from the mature stage and alpha amylose contained a trace of extraneous fatty material, therefore it was either removed or corrected for, in determining the amount of fatty material freed by hydrolysis. The amount of this fatty material increased in the alpha fraction of the soluble polysaccharides as the corn matured. However, there was never as much present in these fractions as there was in alpha amylose. In making the determinations for the fatty material freed by hydrolysis, a flocculent precipitate occurred during the hydrolysis. This was collected

TABLE VI

COMPARISON OF PROPERTIES OF HYDROLYZED ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND HYDROLYZED ALPHA AMYLOSE FROM SWEET CORN STARCH

<table>
<thead>
<tr>
<th>Material</th>
<th>Conversion to Glucose on Hydrolysis %</th>
<th>Specific Rotation of Hydrolyzed Samples at 25° C.</th>
<th>Melting Point of Osazones from Hydrolyzed Samples °C</th>
<th>Fatty Material Liberated by Hydrolysis %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha fraction of soluble polysaccharides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk stage</td>
<td>76.27</td>
<td>36.41</td>
<td>206.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Dough stage</td>
<td>90.23</td>
<td>51.32</td>
<td>204.0</td>
<td>0.03</td>
</tr>
<tr>
<td>Mature stage</td>
<td>91.90</td>
<td>55.25</td>
<td>204.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Alpha amylose</td>
<td>92.25</td>
<td>55.93</td>
<td>204.4</td>
<td>0.79</td>
</tr>
</tbody>
</table>
on a filter and washed free of acid before extracting with ether. The alpha fractions of the soluble polysaccharides from the milk and dough stages contained an abundant amount of this flocculent precipitate after hydrolysis, but when this was collected on a filter and washed it was found to be water soluble.

**Properties of Water Soluble Polysaccharides Obtained from Evergreen Sweet Corn in Milk Stage**

The water soluble polysaccharides from the Evergreen sweet corn were prepared in the same manner as described for the Hopeland sweet corn. The properties of the beta fractions of the soluble polysaccharides were very similar to those of the Hopeland fractions, with the exception that the Evergreen fractions had a higher conductivity.

The properties of the alpha fraction of the soluble polysaccharides from the Evergreen corn were similar to those of the Hopeland alpha fraction in the milk stage, with the exception that the Evergreen material had more ash and more fatty material freed by hydrolysis.

**Discussion**

The data on the beta fraction of the soluble polysaccharides indicate that it is possible to obtain from sweet corn a preparation which will have fairly consistent properties from year to year. The change in properties of this fraction as the corn matures may be due to an increase in the chain length of the compound. Therefore an attempt was made to determine the molecular weights of the different preparations by the cryoscopic method, but it was not possible by this method to obtain consistent results. In comparing the properties of the beta fraction of the soluble polysaccharides with those of beta amylase, it was evident that the physical properties of these two compounds are different. The former was readily soluble in cold water and gave an opalescent solution while the latter was difficultly soluble in cold water and gave a water-clear solution. The beta amylase had a greater viscosity, conductivity, and optical rotation than the beta fraction of the soluble polysaccharides. The chemical data indicate that these two compounds differ only in their physical properties. The specific rotation of the beta amylase was higher than that reported by other investigators (4, 24) working with beta amylase prepared from other kinds of starch.

The solubility of the alpha fraction of the soluble polysaccharides and alpha amylase was similar. Both compounds were readily soluble before electrodialysis; however, after repeated electrodialysis it was exceedingly difficult to get either compound into a stable suspension. Owing to this fact such physical measurements as viscosity, conductivity, and optical rotation would be of doubtful value.
The properties of the two fractions of the water soluble polysaccharides which have been investigated are not similar to any of the dextrins resulting from starch hydrolysis, as reported by Samec (21). Some dextrins resulting from direct hydrolysis of starch have been reported by Baker (3) and by Lintner and Düll (15) as having specific rotations which were very similar to that of the beta fraction of the soluble polysaccharides; however, the other properties reported by these investigators for their dextrin preparations were quite different from the properties of the alpha or beta fractions of the soluble polysaccharides. Link (14) isolated and purified a dextrin from the radicle and plumule of the young corn plant. This dextrin was not similar to either fraction of the soluble polysaccharides found in sweet corn endosperm.

Lampe and Meyers (13) have studied the development of the endosperm of sweet corn microchemically, and report that globules first form and that carbohydrate grains which give characteristic starch reactions may or may not form within these globules. They advance the theory that these globules contain the water soluble polysaccharides. This idea was later given more strength by Lampe (12). These investigators also report that they found no evidence of a reversal or a hydrolysis after the carbohydrate grains were once formed.

In the light of these recorded observations and the fact that the present investigation shows that the water soluble polysaccharides contain two fractions which in some respects are similar to sweet corn starch components, it is possible that the water soluble polysaccharides present in sweet corn are the units for the formation of starch grains.

Summary

1. The water soluble polysaccharides present in sweet corn endosperm were isolated and divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions have been tentatively named alpha and beta, respectively.

2. The physical and chemical properties of these fractions of the soluble polysaccharides from corn in the milk, dough, and mature stages were compared with each other and with alpha and beta amylose prepared from sweet corn starch.

3. As the corn matured many of the properties of the alpha and beta fractions of the soluble polysaccharides approached those of alpha and beta amylose of starch but they departed widely from those that have been described for dextrins.
The writer wishes to acknowledge his indebtedness to Dr. C. O. Appleman for the assistance and constructive criticism rendered in this investigation, and to Dr. C. M. Conrad for his assistance and counsel.

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