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During an outbreak of spoilage in commercially prepared salad dressing, the only organism that was isolated proved to be a species of Lactobacillus. The evidence pointed to this lactic acid bacillus as responsible for the spoilage. Although this report will deal principally with the physiology and classification of *Lactobacillus fructivorans* sp. nov., some remarks concerning the spoilage may be of interest.

The commercial preparation of mayonnaise and various types of salad dressings has become an important food industry in the United States. Mayonnaise is an emulsion composed chiefly of vegetable oil, egg yolk, water, and vinegar to which certain condiments are added. The composition of this product is subject to certain restrictions under food laws. Other salad dressings are made from similar ingredients to which a variety of substances such as pickles, olives, meat, sugar and starch may be added. There do not appear to be definite standards in regard to the composition or method of preparation and preservation of these types of salad dressings. Large numbers of bacteria which may be present in the ingredients are practically all destroyed by heating during the manufacture of the dressing. Bacterial spoilage by any surviving organisms in the finished product does not usually occur owing to the concentration of acetic or lactic acid employed. Other undesirable changes in salad dressings, such as discolorations, changes in consistency, emulsion break-down, and especially the development of rancidity present difficulties to the manufacturers.

The literature relating to bacterial spoilage of salad dressings is not extensive. Pederson (1930) studied an outbreak of spoilage due to an aerobic spore forming organism. Spoilage due to similar types of bacteria had been reported by other investigators and their findings were reviewed by Pederson.

**THE FERMENTED SALAD DRESSING**

Samples of spoiled salad dressing were submitted for examination. The manufacturer was inexperienced with this type of product, and the spoilage was excessive; about one-fifth of the first five thousand cases were returned as undergoing spoilage. The flavor and odor of the spoiled product were not abnormal; the reaction was found to be pH 4.1, which is not different from that to be expected in this type of product. A slight lumpiness was present. The development of gas in the product, the only evidence of spoilage, took place very slowly. On opening the glass con-
Stainers a "bubbling over" of a portion of the contents occurred. No marked gas pressure was developed by any of the fermented samples.

Stained preparations of the spoiled product revealed slender gram-positive rods occurring in chains. Morphologically the organisms were of one type; they were not uniformly distributed, but were present primarily in clusters of intertwined chains. No spores were observed.

**ISOLATION AND STUDY**

Attempted isolations of the causative organism were at first unsuccessful. Aerobic and anaerobic platings on milk powder agar, veal infusion agar and plain agar showed no growth. Subsequent platings from two samples were made on tomato juice agar (ordinary nutrient agar containing about 20 per cent of neutralized tomato juice). The number of colonies gave a plate count of about 50,000 bacteria per gram of spoiled salad dressing. Growth was much slower and there were fewer colonies on the aerobic plates than on those incubated in an atmosphere containing carbon dioxide. The difficulty experienced in obtaining growth on ordinary media followed by the successful use of tomato juice agar is substantially the experience of Mickle and Breed (1925) in isolating *Lactobacillus lycopersici* from spoiled catsup. These authors state, "... little success attended efforts of isolation with autolysed yeast broths, sodium oleate media, casein digest or milk powder agars, ... even though the hydrogen ion concentration was varied..."

The isolated organisms were of one type, being gram-positive, non-spore forming rods. In cultural behavior, particularly in forming large entire subsurface colonies and distinctly dome-shaped surface colonies, the organisms more closely resembled certain of the propionic acid bacteria than the lactobacilli. Other interesting characteristics were noted which suggested the advisability of a detailed study.

**DESCRIPTION OF LACTOBACILLUS FRUCTIVORANS sp. nov.**

In the following description, the broth, agar, and gelatin media contained 20 per cent of neutralized filtered tomato juice unless otherwise stated.

**MORPHOLOGY**

Grown on agar at 30° C. in an atmosphere to which approximately 50 per cent carbon dioxide is added the rods are from 0.4 to 0.8µ by 1.5 to 4µ. The chains of cells show a distinct "looping" characteristic, often turning upon themselves. The individual cells may also be curved. (Fig. 1.) When the organism is grown aerobically, this turning characteristic is much more pronounced so that the cells occur principally in tightly massed groups (Fig. 4). In broth the cells occur as slender rods in chains and the swelling and curving tendencies are not pronounced. Long filaments occur frequently. The cells are non-motile, stain evenly and are gram-positive.

**CULTURAL CHARACTERS**

Agar slant. Aerobic growth is scanty. Heavy inoculation leads to a beaded white growth. (Fig. 8b.) Under anaerobic conditions at 30° C. an abundant growth is obtained within 48 hours. Equal volumes of air
PHYSIOLOGY OF LACTOBACILLUS FRUCTIVORANS SP. NOV.

and nitrogen or air and carbon dioxide allow good development. The growth is smooth, white, butyrous and opaque.

Agar stab and agar shake. There is an optimum zone of growth in the vicinity of 2 cm. below the surface (Fig. 9a). When not crowded the colonies in an agar shake may attain a diameter of 4 mm. In glucose tomato agar shake culture splitting of the agar due to gas development usually appears, but is never extensive.

Agar colonies. Surface colonies are circular with a diameter from 1 to 3 mm., after 4 days at 30° C., in a 50 per cent CO₂ atmosphere. The colonies are raised, smooth, white, amorphous and entire. In a humid atmosphere with a slightly moist agar surface the colony edge may be filamentous (a young colony is shown in fig. 6). On a dry surface, the edge is entire and the colony was be “dome-shaped.” The sub-surface colonies are lens-shaped and entire.

Gelatin. No liquefaction.

Broth. In broth, brought to a boil before inoculation, there is a “snowflake” type of growth to within one centimeter of the surface. This growth settles to the bottom or adheres to the wall of the tube (Fig. 10c). If the broth is not boiled before inoculation quite a different cultural characteristic is observed, the growth develops more slowly and is confined at the bottom to a depth of about 5 mm. The supernatant liquid is clear.

PHYSIOLOGICAL CHARACTERS

Gas production. Glucose broth fermentation in Smith tubes leads to the development of gas amounting to about 10 per cent of the closed arm after 7 days at 30° C.

Litmus milk. The organism remains alive for some time and there may be slow growth. Milk remains unchanged. The addition of levulose or tomato juice leads to coagulation of the milk.


Catalase production. Negative.

Temperature relationships. Optimum 25° to 30° C., growth at 37° C., no growth at 40° C. The cells die rapidly at 65° C.

Dissimilation of carbohydrates. Yeast extract 0.2 per cent, peptone 0.5 per cent, K₂HPO₄, 0.1 per cent, neutralized tomato juice 5 per cent, carbohydrate 1 per cent. Acid from glucose and levulose.

No acid from: Mannose, galactose, sucrose, maltose, lactose, trehalose, xylose, arabinose, a-methyl glucoside, inulin, dextrin, glycerol, raffinose, salicin, starch, dulcitol, sorbitol.

Considering the foregoing description, as well as the dissimilative characteristics reported in the following pages, which show that lactic acid but no propionic acid is produced from glucose, the organism should be placed in the genus Lactobacillus. It is not like any of the described species and is distinctive in that with the exception of glucose and levulose it fails to ferment the commonly employed carbohydrates. Levulose is much more strongly attacked than glucose.

Lactobacillus fructivorans is suggested as a name to designate this new species because of its preference for fruit sugar.
OBSERVATIONS OF THE NUTRITIVE REQUIREMENTS OF L. FRUCTIVORANS

The organism does not grow perceptibly in peptone or in yeast extract broth; upon addition of tomato juice good growth is obtained. Although no special attempt was made to determine the constituent in tomato juice responsible for this stimulation of growth some observations with tomato juice and other vegetable and plant extracts may be of interest.

Pure unneutralized tomato juice supports a luxuriant growth of the organism and as low as two per cent of tomato juice in peptone broth is stimulating. Tomato juice which has been fermented with Aerobacter aerogenes, filtered and re-sterilized, still retains the growth promoting property.

The juices of apples and pears added to peptone broth constitute satisfactory media, although they are not as stimulating as tomato juice. Aqueous extracts of carrot, cabbage, soy bean and potato were added to peptone broth, but none exhibited an appreciable stimulation of L. fructivorans. Similar results were obtained with 0.5 per cent concentrations of the sodium salts of malic, citric and tartaric acids in peptone broth.

Difficulty was encountered in the choice of a medium for study of the physiological characteristics of L. fructivorans. The importance of a proper nitrogen source in such studies has been stressed by Orla-Jensen (1919) and by Sadler and Eagles (1932). Undoubtedly this is an important consideration, for apparently inconsistent results may be obtained by changing the nitrogen source. However, in the case of certain lactobacilli, a preference for certain types of carbohydrates has been observed. In this connection Müller-Thurgau and Osterwalder (1912) have shown that with Bact. gracile, if glucose and malic acid are present in a given medium, the malic acid is utilized first, apparently in preference to the glucose. Certain bacteria are known to show a predilection for levulose; this preference is further illustrated in the present case. In a suitable medium, glucose is fermented, yet in glucose-peptone broth no appreciable growth or fermentation of the sugar occurs. On the other hand, peptone broth containing levulose is a satisfactory medium, although improved upon the addition of tomato juice. Müller-Thurgau (1908) reported similar observations when employing a related species, Bact. mannitopoeum. He found that upon the addition of various carbohydrates and salts of organic acids to a basal medium of peptone, 1 per cent; K₂HPO₄, 0.2 percent; and NaCl 0.5 per cent, that growth occurred only in the presence of sucrose or levulose. There was no growth in the presence of glucose or maltose even though these sugars are fermented by Bact. mannitopoeum when the basal medium contains yeast extract and malic acid. From these observations, and from some results in the studies reported below, it appears that the "mannitol forming" organisms in the genus Lactobacillus exhibit a predeliction for levulose, and in one case were found to utilize an organic acid in preference to glucose.

COMPARATIVE STUDIES ON L. FRUCTIVORANS AND L. GRACILIS

Lactobacillus fructivorans belongs to the group of mannitol-forming bacteria reported in studies on spoiled wines by various investigators.
Pederson (1929) has studied organisms representing a number of species of Lactobacillus. The only previously described species which resembles *L. fructivorans* is one described by Müller-Thurgau (1908), as *Bacterium gracile*. Further description of *Bact. gracile* was recorded by Müller-Thurgau and Osterwalder in 191 and 1917. Additional studies on *Bact. gracile* have not appeared, although the organism is referred to as *L. gracile* in Bergey’s Manual (1930). *Lactobacillus gracilis* is the correct form.

Our appreciation is expressed to Dr. Osterwalder for sending us a culture of *L. gracilis*.

*L. fructivorans* and *L. gracilis* differ in size (compare the preparations from agar and broth shown in figures 1 and 3; 5 and 7). The looping tendency of the chains of cells is characteristic of both. On morphological grounds, *L. gracilis* should belong to the genus *Leuconostoc* as described by Hucker and Pederson (1931), and the original description of the organism does not conflict with this allocation.

Culturally, the two species are similar. The same type of milk-white colony is characteristic of both. The oxygen relationships, nutritive requirements, and temperature relationships of the two species are similar. The colonies of *L. fructivorans* are as a rule larger, a difference in structure, apparent only under the microscope (Figs. 2 and 6), is related to the differences in cell size. This fact may partly explain the differences in broth cultures (Fig. 10).

Both species produce inactive lactic acid.

The two organisms may be differentiated on the basis of fermentation reactions. Trehalose and α-methyl glucoside are fermented by *L. gracilis* but not by *L. fructivorans*.

**PRODUCTS OF DISSIMILATION OF GLUCOSE AND LEVULOSE**

**QUANTITATIVE METHODS FOR DETERMINING PRODUCTS OF FERMENTATION**

Lactic Acid: An aliquot of the fermented liquor was evaporated to 10 cc., acidified to congo-red paper and taken up in anhydrous sodium sulfate. The resulting dry crumbly mixture was placed in a thimble and continuously extracted for eight hours. The ether was distilled off and the residue brought to a volume of 100 cc. with water. A 10 cc. fraction was neutralized to phenolphthalein with sodium hydroxide, boiled three minutes with an excess of alkali and again brought to neutrality. The lactic acid was then determined by the method of Friedemann and Kendall (1929).

Ethyl alcohol was determined by the method described by Stahly, Osburn, and Werkman (1934).

Acetic acid was determined by steam distilling and partitioning the distillate with ethyl ether according to the method of Osburn, Wood, and Werkman (1933).

Carbon dioxide was caught in a Bowen potash bulb and weighed.

Mannitol was formed only in solutions containing levulose. Levulose is soluble and mannitol insoluble in ether, making possible the separation from interfering substances by ether extraction.

The residue from the ether extraction was extracted eight hours with 95 per cent alcohol. The alcohol was evaporated and the mannitol dissolved in water and made up to 100 cc. The determination was made by the method suggested by Smit (1914).
Qualitatively, the products obtained from the fermentation of glucose by both species were the same, i.e., ethyl alcohol, lactic acid, acetic acid, and carbon dioxide. These products and in addition mannitol were formed from levulose.

For the quantitative determination of the products of fermentation a medium consisting of one per cent peptone, five per cent tomato juice, 0.2 per cent potassium phosphate (dibasic) and two per cent sugar was prepared. One liter of the medium was placed in two-liter Erlenmeyer flasks. The sugar was sterilized separately and added aseptically. Ten cubic centimeters of a five day culture of the organism in question were used as inoculum. All fermentations were incubated at 30° C. for 21 days. During fermentation, nitrogen was continuously bubbled through the medium.

In order to compensate, in a measure, for the acid contained in the tomato juice added to the medium, the yields obtained by analysis of an uninoculated flask were subtracted from those obtained in the fermented medium.

Table 1 shows the yields of products in the anaerobic fermentation of glucose and levulose by the two species. No propionic, succinic, or formic acid was found in any of the fermentations.

The data in table 1 emphasize the utilization of levulose. A review of the literature indicates that this is characteristic of the lactic acid organisms producing mannitol from levulose. Levulose functions as both hydrogen acceptor and (after splitting) as donator.

It is seen that L. fructivorans is more active in fermenting glucose and levulose than L. gracilis. The former fermented 38.5 millimols of glucose and 113.8 millimols of levulose while L. gracilis fermented only 10 millimols of glucose and 89.3 millimols of levulose. The difference in the quantity of sugar fermented by the two species may, in part, be due to a difference in medium required for optimal growth.

It has been noted that these organisms are micro-aerophilic. This fact is further emphasized by an experiment with the same medium as was used above. Air passing first through a bead tower containing potassium hydroxide, was bubbled through the medium for five hours at the beginning of the fermentation to remove carbon dioxide present in the system. It was again aerated after two weeks and at the conclusion of the fermentation which continued thirty days. The results of this experiment are presented in table 2.

Comparison of aerobic and anaerobic fermentations shows that greater utilization of glucose takes place under aerobic conditions. This, in part, may be due to the longer period of fermentation. The greater portion of fermentation takes place, however, in the first few days as shown by Weinstein and Rettger (1932) and Fred, Peterson and Davenport (1920). The longer period of fermentation is not likely to influence the sugar utilization to the extent shown by the data in tables 1 and 2.

The results show that L. fructivorans is a hetero-fermentative lactic acid organism and is related to those described by Pederson (1929), Fred, Peterson and co-workers (1919, 1921), Gayon and Dubourg (1901), von Steenberg (1920), and Müller-Thurgau and Osterwalder (1912).
**TABLE 1. Anaerobic dissimilation of glucose and levulose by L. gracilis and L. fructivorans**

<table>
<thead>
<tr>
<th>Sugar used</th>
<th>L. fructivorans</th>
<th>L. gracilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Levulose</td>
</tr>
<tr>
<td></td>
<td>Milli-mols</td>
<td>Percent-age of sugar</td>
</tr>
<tr>
<td>Glucose</td>
<td>38.5</td>
<td>113.3</td>
</tr>
<tr>
<td>Levulose</td>
<td>37.8</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td>67.3</td>
<td>52.4</td>
</tr>
<tr>
<td></td>
<td>12.25</td>
<td>12.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>47.55</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>26.7</td>
<td>28.6</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>34.5</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>82.5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

**TABLE 2. Aerobic dissimilation of glucose by L. fructivorans and L. gracilis**

<table>
<thead>
<tr>
<th>Sugar used</th>
<th>L. fructivorans</th>
<th>L. gracilis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Millimols</td>
<td>Percentage of carbon in sugar</td>
</tr>
<tr>
<td>Glucose</td>
<td>47.55</td>
<td>57.3</td>
</tr>
<tr>
<td>Levulose</td>
<td>40.8</td>
<td>26.6</td>
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<td></td>
<td>28.6</td>
<td>9.4</td>
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<td></td>
<td>36.1</td>
<td>25.7</td>
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<tr>
<td>Mannitol</td>
<td>282.5</td>
<td>36.1</td>
</tr>
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<td></td>
<td>60.2</td>
<td>22.0</td>
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</tbody>
</table>
**SUMMARY**

*Latobacillus fructivorans* sp. nov., isolated from spoiled salad dressing, is described and compared with *L. gracilis* from which it was morphologically and culturally differentiated.

*L. fructivorans* and *L. gracilis* belong to the group of heterofermentative lactic acid bacteria. Dissimilation of glucose leads to the production of inactive lactic acid, acetic acid, ethyl alcohol, and CO₂ and in addition mannitol is formed from levulose.

Photo-micrographs of *L. gracilis* (*Bact. gracile*) agree with those in the original descriptions and indicate that it is of the Leuconostoc type and not a species of Lactobacillus as described by Bergey (1930).

Dissimilation of levulose by both species differs from that of glucose in that the nature and quantitative relationships of the reduced products are changed. Part of the levulose acts as a hydrogen acceptor to be reduced to mannitol. The reduction of levulose is compensated for by greatly lowered yields of other reduction products, that is, ethyl alcohol.

The mannitol-forming members of the genus Lactobacillus are not readily differentiated from species of Leuconostoc Hucker and Pederson (1931). The two types act similarly in the dissimilation of glucose and levulose and agree in other respects, such as in cultural appearance, temperature requirements, and habitat. Since the cells of some Leuconostoc species may elongate into a rod the morphological distinction cannot be maintained.

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**MÜLLER-THURGAU, H.**
PHYSIOLOGY OF LACTOBACILLUS FRUCTIVORANS SP. NOV.

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PLATE I

Fig. 1. *Lactobacillus fructivorans* grown on tomato juice agar at 30° C. in an atmosphere of 50 per cent carbon dioxide. Gram stain x 1080.

Fig. 2. Six day colony of *L. gracilis* on tomato juice agar at 30° C. and in an atmosphere of 50 per cent carbon dioxide. Unstained. x 60.

Fig. 3. *L. gracilis* grown on tomato juice agar at 30° C. in an atmosphere of 50 per cent carbon dioxide. Gram stain x 1080.

Fig. 4. *L. fructivorans* grown aerobically on tomato juice agar at 30° C. Eight day culture. Nigrosine preparation. x 1080.

Fig. 5. *L. gracilis* grown in tomato juice broth at 30° C. Gram stain x 1080.

Fig. 6. Six day colony of *L. fructivorans* on tomato juice agar at 30° C. and in an atmosphere of 50 per cent carbon dioxide. Unstained. x 60.

Fig. 7. *L. fructivorans* grown in tomato juice at 30° C. Six day culture (then held two days at room temperature). Nigrosine preparation. x 1080.

Fig. 8. a. *L. gracilis* in glucose tomato juice agar at 30° C. in an atmosphere of 50 per cent carbon dioxide. Unstained.

b. *L. fructivorans* as under a.

Fig. 9. a. *L. gracilis* in glucose tomato juice agar at 30° C. Showing two growth zones. Six day cultures.

b. *L. fructivorans* in glucose tomato juice agar at 30° C. Six day culture. Showing growth zone.

Fig. 10. a. *L. gracilis* in tomato juice broth at 30° C. Six day culture.

b. Uninoculated control tube of tomato juice broth.

c. *L. fructivorans* in tomato juice broth at 30° C. Six day culture. Showing growth zone and also precipitated organisms.
PHYSIOLOGY OF LACTOBACILLUS FRUCTIVORANS SP. NOV.
ON THE TOTAL BLOOD (HEMOLYMPH) CELL COUNT OF THE FIELD CRICKET, GRYLLUS ASSIMILIS PENNSYLVANICUS BURM.¹

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Total blood cell counts of vertebrates, particularly of mammals, have been reported frequently in the literature, usually in connection with physiological investigations. On the other hand, total blood cell counts of invertebrates, especially of insects, appear to have been recorded very infrequently. Hardy (1892), using a Gower’s hemacytometer, obtained an average total count of 286 cells per mm³ of blood for the crustacean, Astacus, the values ranging from 250 to 400 cells per mm³. Blood was used both fresh and after osmic acid vapor fixation. Tillyard (1917) roughly estimated that an entire dragonfly larva of very small size must contain only two or three hundred cells, and suggested that the number may be increased with each succeeding ecdysis. Haber (1926) estimated that there are about 1,400 to 2,500 cells per medium sized drop of blood from the cockroach, Blattella germanica Linn. Landois and Landois (1865) state that “by general estimation” the blood cells of the larva of Smerinthus populi remain at a constant level, reaching their highest count shortly before the pupal stage and subsequently decreasing to a minimum during metamorphosis; they do not present figures for total cell counts. Hollande (1930) states that Aghar (1928)²; in his doctorate thesis, presents values for insect total blood cell counts, finding respectively 12,000 and 10,000 cells per mm³ of blood from Pieris brassicae L. and Aporia crataegi L. Yeager and Tauber (1932) obtained an average total cell count of approximately 30,000 cells per mm³ of blood from the roach, Periplaneta fuliginosa Serville, individual counts ranging from 16,916 to 65,355 cells per mm³ blood. They also report (1933) an average total cell count of about 30,000 cells per mm³ blood from the roach Periplaneta orientalis L.; the values ranging from 14,400 to 57,600. Fischer (1934) found the range for total blood cell counts for P. orientalis to be from about 9,000 to 84,000 cells per mm³ of blood, with an average of approximately 34,000.

The present paper contains an analysis of 220 total blood cell counts obtained from the common field cricket, Gryllus assimilis pennsylvanicus Burm.

ANIMALS

The field crickets used for the total blood cell counts reported here were randomly collected at Ames, Iowa, in the months of June, July, September, October, and November; that is, from their first appearance in the spring until their disappearance in the fall. Only the larger adults

¹This report is part of work being done under a grant from the Rockefeller Fluid Research Fund of Iowa State College.

²The authors have not had access to Aghar’s original publication.
and nymphs were used for the counts, which were made as soon after collection as practicable. While kept in the laboratory the crickets were supplied with water and food (banana, oat sprouts, and grass). Specimens collected in spring and summer appeared to remain in good condition in the laboratory, but many, especially the adults, caught in the late fall often died after being caged indoors for a few days. Whether this mortality was due to natural seasonal causes is not known, but since the spring animals could be kept for several weeks in good condition, this factor probably was the important one in producing the high death rate in the late fall.

**METHOD**

Counts recorded here were obtained with a double chamber hemacytometer, customarily used for making total blood cell counts of vertebrates. A specially made diluting pipette, by means of which 1.22 mm³ of blood could be diluted 150 times, was employed in order that a minute quantity of the rapidly coagulating blood of the insect might be quickly diluted and cell coagulation thereby prevented. The dilution fluid consisted of 0.081 M NaCl, 0.002 M KCl, 0.001 M CaCl₂, 0.005 per cent gentian violet, and 0.125 per cent glacial acetic acid. Otherwise, the procedure was the same as that usually employed with vertebrate blood.

The blood sample was obtained from an antenna, cut off near its base. All but six of the animals collected in September, October and November were subjected to glacial acetic acid vapor to prevent cell coagulation (Shull, Riley, Richardson, 1932). An animal so treated was suspended by a thread for five to ten minutes, depending on the reactions of the animal, above the surface of 25 cc. of glacial acetic acid in a closed 250 cc. wide mouth bottle. The other animals were employed without acetic acid treatment.

**RESULTS**

Total blood cell counts have been obtained from 220 different individuals of *Gryllus assimilis pennsylvanicus* Burm. The values are widely distributed with a mean of 70,118 and a range from approximately 15,000 to 275,000 cells per mm³ of blood. It is not necessary to tabulate here the individual values of the counts since the data can be better illustrated by the frequency distributions employed in the analysis of these results.

When the counts are arranged according to the sex or stage of development of the animals from which they were obtained, the various group averages are as follows: mature and nymphal males (73 animals), 64,020; mature and nymphal females (147 animals), 73,147; mature males and females (197 animals), 73,099; nymphs of both sexes (23 animals), 44,589; mature males (63 animals), 69,886; mature females (134 animals), 74,609; male nymphs (10 animals), 27,064; female nymphs (13 animals), 58,071. It is seen that the average for all females is higher than the average for all males; that the average for the mature animals is higher than the average for the nymphs; that the average for the mature females is higher than that of the mature males; and that the average for the nymphal females is considerably higher than that of the nymphal males. These figures indicate that, in general, the average total blood cell count for the female crickets tends to be higher than that for the males, although the modes for the two sexes coincide (Fig. 2).
If the individual values of the 220 total blood cell counts are grouped with class intervals of 20,000 cells per mm$^3$ of blood, their skewed frequency distribution is that shown by the polygons of figure 1, A. With this grouping, the distribution is very nearly that represented by Pearson’s Type III curve (see Elderton, 1906) as indicated by the values of the criteria $\beta_1$, $\beta_2$, $\kappa$, provided that the single value of 275,490 be discarded (a procedure justified by Chauvenet’s criterion). From an experimental standpoint, a class interval of 20,000 cells per mm$^3$ of blood is too large to be of much practical use, for it implies too great a counting error; also, its size tends to obscure certain characteristics of the distribution that appear with a 10,000 cells per mm$^3$ class interval. Nevertheless, it is of interest that the distribution from 10,000 to 110,000 is almost symmetrical, with nearly normal distribution.

Fig. 1. A—Frequency distribution of 220 total blood (hemolymph) cell counts of the field cricket, *Gryllus assimilis pennsylvanicus*, with class interval of 20,000 cells per mm$^3$ of blood. B—Frequency distribution of the same series of counts but with a class interval of 10,000. The average for the series is 70,118 cells per mm$^3$ of blood. The line connecting the circles is a theoretical normal distribution curve calculated for all counts of value 110,000 or less. The line connecting the crosses is a theoretical Poisson series curve calculated for the series of 218 counts of value 200,000 cells per mm$^3$ of blood or less. The polygons represent the observed distribution.

The authors wish to thank Dr. E. R. Smith and Mr. Cuthbert C. Hurd, of the Mathematics Department, for their kindly help in this connection.
In figure 1, B, a class interval of 10,000 cells per mm$^3$ of blood is used. The nearly perfect symmetry of the 10,000 to 110,000 range is broken partly by the low frequency of the 65,000 polygon. With that exception, this limited range tends to be normally distributed, as is shown by the moderately good fit of the theoretical normal (Type VII) curve (circles), calculated on the basis of only those counts of 110,000 cells per mm$^3$ or less. It is obvious, however, that the entire distribution is not one of pure type but is a complex one, skewed toward the higher counts and with modes at 55,000, 75,000, and 125,000 cells per mm$^3$. [In figure 1, B, the crosses represent a Poisson series (see R. A. Fischer, p. 55) calculated for 218 counts of 200,000 cells per mm$^3$ or less, using a mean of 63,394. Although the theoretical values, in general, follow the form of the observed distribution, it is obvious that the fit is not entirely satisfactory.] The question arises, what are the factors tending to produce this trimodality and to skew the distribution of these counts? While a complete answer to this question must await further investigation, interesting leads may be obtained by additional analysis of the data.

The entire series of counts may be divided into three groups: counts from females (nymphs and adults), counts from males (nymphs and adults), and counts from nymphs of both sexes taken together. The frequency distributions for the three groups are shown in figure 2. Each distribution appears as a skewed, trimodal curve, of the same type as that of the whole series (Fig. 1, B), with modes at 55,000, 75,000, and 125,000 (females); at 45,000, 75,000, and 125,000 (males); and at 25,000, 75,000 and 115,000 (nymphs) cells per mm$^3$. From this it would appear that the trimodality and asymmetry of the entire series (Fig. 1, B) are not due to
TOTAL BLOOD CELL COUNTS: CRICKET

differences of distribution in male and female nymphs, males and fe-
males; or in other words, that they are not due to maleness, femaleness,
or a general growth factor (difference between nympha! and imaginal
stages). It is of interest, however, that all counts above 120,000 cells per
mm$^3$ were obtained from mature insects. Furthermore, all counts be-
tween 120,000 and 145,000 are from either mature males or mature fe-
amales, and all the remaining figures above 145,000 cells per mm$^3$ of blood
are from mature female crickets alone. These high counts in the distri-
butions of the two sexes, particularly in the female, contribute largely to
the asymmetry of the entire series. Also, the difference between the
maximal limits of the counts from nymphs and adults may contribute,
partially, to the existence of the skewness of the entire distribution, but
the number of nymphs counted in this study is small (23) and does not
warrant definite conclusions.

![Graph showing total blood cell counts among animals collected in June
(Ju.), July (Jy.), September (S.), October (O.), and November (N.). The means
are 73,073 (June), 83,710 (July), 59,552 (September), 60,144 (October), and 71,039
(October). The two highest counts (223,245 and 275,490) are omitted.]

In a similar way, the total series of counts may be grouped according
to the month during which the animals were collected. Figure 3 shows
the frequency distributions of five groups of crickets collected in the
months of June, July, September, October, and November, respectively.
With the exception of September, during which only a few animals were
collected, the monthly frequency distributions are trimodal, skewed to-
ward the higher counts, and, hence, essentially like the distribution of
the entire series (Fig. 1, B). The values at the various modes are shown
in figure 3. The modes at 25,000, 95,000, and 115,000 may be of question-
able significance. From this it is seen that the trimodality and skewness of the entire distribution are also characteristic of the monthly frequency distributions and, consequently, it would appear that these properties of the entire distribution (Fig. 1, B) are not due to collecting the animals at various times of the year.

The number of mature males, mature females, and male and female nymphs collected in the different months are as follows:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>19</td>
<td>31</td>
<td>2</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Females</td>
<td>7</td>
<td>41</td>
<td>4</td>
<td>69</td>
<td>13</td>
</tr>
<tr>
<td>Nymphs</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

The frequency distributions of counts from acetic acid treated animals are shown in figure 4. The counts from the acid treated crickets tend to be somewhat lower than those from normal, untreated animals, the former group having an average of 57,735 cells per mm$^3$ of blood and modes at 55,000, 75,000, and 125,000, while the counts from the untreated animals are distributed with an average of 82,380 cells per mm$^3$ and with modes at 55,000 and 75,000; both distributions are skewed toward the higher counts. The authors have found from experience that in making insect total blood cell counts, the greater the degree of blood coagulation the greater is the number of cells lost to the cell coagulum and the fewer are the cells in the counting chamber, and hence the lower is the total cell count. Fixation of the cells by heat or acetic acid vapor prevents the formation of a cell coagulum, prevents the loss of cells from the counting chamber, and prevents the total cell count from appearing less than its true value, in so far as cell coagulation is concerned. If the use of acetic acid vapor were to affect the distribution, it would be expected to make the counts somewhat higher than those from the untreated animals. The distributions in figure 4, however, show the opposite effect; also, it would seem very probable that the acid is not a factor producing the trimodality and skewness of the entire distribution (Fig. 1, B). This conclusion is supported by the work of Fischer (1934), who found that the use of acetic acid vapor as an anticoagulant results in total blood cell counts significantly higher than counts made on blood from normal, untreated animals, and who also noted that in the latter cases there was always evidence of cell coagulation in the counting chamber.

It has been suggested by Allard (1929) that some crickets may have more than one brood per annum, some animals overwintering in the egg stage, some in the nymphal stage. If this is the case, in the species used in this study, the large nymphs and imagos collected in June and July would represent one brood (overwintering in the nymphal stage) while

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4In the present work, clumps of agglutinated cells appeared rarely in the counting chamber when blood from normal, untreated animals was used; this is due to the fact that the authors employed a specially made micro diluting pipette, the use of which permits very rapid dilution of only 1.52 mm$^3$ of blood and thereby prevents the occurrence of cell coagulation. If, as infrequently occurred, evidence of cell coagulation was noted in the pipette or the counting chamber, the preparation was discarded and the count not made. The low counts obtained by Fischer from untreated animals are very probably due to the use of an ordinary white blood cell diluting pipette, which, requiring a larger amount of blood, permits the occurrence of cell coagulation.
those captured in September, October, and November would represent another brood (overwintering in the egg stage). Examination of the frequency distributions for June, July, October, and November (Fig. 3) shows that they all tend to be trimodal and asymmetrical toward the

**Fig. 4.** Distribution of total blood cell counts among (A) animals treated with acetic acid vapor as anticoagulant and (W) normal, untreated animals. The means are 57,735 (A) and 82,380 (W) cells per mm³ of blood. This difference is not to be considered due to the use of acid. Compare with Fig. 5. See text. The two highest counts (223,245 and 275,490) are omitted.

**Fig. 5.** Distributions of total blood cell counts among animals collected in (S) early summer (June-July) and (F) fall (September-October-November). The means are 79,479 (S) and 61,414 (F) cells per mm³ of blood. Compare with Fig. 4. Higher counts, thereby resembling the entire distribution (Fig. 1, B), whose characteristics of trimodality and skewness, therefore, are not attributable to the month during which the animals were collected.
However, if the counts from animals collected in June and July are grouped together and separated from the group of animals made up of those collected in September, October, and November, the distributions of the two groups take the form shown in Figure 5. The mean for the June-July group is 79,479; for the September-October-November group, 61,414. The difference between the two means is 18,065 with a standard error of ± 4,780. This difference is significant since it is more than twice its standard error (see R. A. Fischer, p. 101 and p. 105). The June-July and the September-October-November distributions are similar, respectively, to the distributions from normal, untreated animals and from acetic acid vapor treated animals (Fig. 4), due to the fact that all but six of the fall animals were treated with acid vapor before making the count. The question therefore arises, do the means of the June-July and September-October-November distributions differ because of the use of acetic acid vapor or because of other factors related to the season during which the animals were collected, as, for example, to the existence of two different seasonal broods? It has already been indicated that such a difference is contrary to the expected effect of the use of acetic acid vapor as an anticoagulant. This greatly increases the probability that the difference between the two means may be due to the local existence of two different broods of this cricket, even though the two broods would seem to be similar with respect to the general characteristics of their total blood cell count distributions.

**DISCUSSION**

The analysis of counts just given indicates that the skewness and trimodality of the entire frequency distribution are not due to maleness, femaleness, general growth stage (i.e., difference between nymph and imago), to the use of acetic acid vapor as an anti-coagulant, to the month during which the animals were collected, or to the existence of two different seasonal broods of this cricket. Although the distributions of the counts from males, females, and nymphs all possess a common characteristic in being skewed toward the higher counts (Fig. 2), the counts above 145,000 cells per mm$^3$ of blood, all of which were obtained from mature female crickets, make the asymmetry of the entire distribution more prominent (Fig. 1, B).

Usually nothing was known of the instar, proximity to molting or to oviposition periods, or to other special physiological and pathological conditions which might occur in a given animal at the time of observation, but in several cases a few records of this kind were obtained, as indicated below:

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Total count (Cells/mm$^3$ blood)</th>
<th>Record of condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>489</td>
<td>223,245</td>
<td>Parasitized, Gordius larvae</td>
</tr>
<tr>
<td>488</td>
<td>125,080</td>
<td>&quot;</td>
</tr>
<tr>
<td>330</td>
<td>106,493</td>
<td>Just after molting</td>
</tr>
<tr>
<td>267</td>
<td>120,625</td>
<td>During molt</td>
</tr>
<tr>
<td>266</td>
<td>118,328</td>
<td>Just before molting (1½ hrs.)</td>
</tr>
<tr>
<td>418</td>
<td>199,995</td>
<td>Ovipositing</td>
</tr>
<tr>
<td>433</td>
<td>275,490</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

In none of the cases above is the total blood cell count low. On the basis of these observations, it may be tentatively suggested that the skewness of the entire frequency distribution toward the higher counts is perhaps
due in part to the occurrence of excessively high total blood cell counts
in animals that are in certain physiological or pathological states brought
about by conditions such as ecdysis, oviposition, and parasitism. Although
the possibility of this suggestion is not lessened by the fact that the fre-
quency distribution tends to be symmetrical in the range from 10,000 to
110,000 cells per mm$^3$ of blood, the proof depends upon further investiga-
tion definitely showing the relationship between total blood cell count
and ecdysis, oviposition, parasitism, or other special physiological or path-
ological conditions of the animal. Tillyard (1917) has suggested that the
number of blood corpuscles in a dragonfly larva appeared to increase
with each succeeding ecdysis; and Landois and Landois (1865) have
stated that the number of blood cells in the larva of Smerinthus populi
varied at different stages in this insect’s life history.

Normally there are several circulatory and nutritional factors which
would tend to increase the range of distribution of insect total blood cell
counts. (1) The insect possesses an open type of circulatory system in
which the blood flows into large sinuses and hemocoelic cavities and,
presumably, comes into direct contact with most of the animal’s tissues.
Circulation may be sluggish, and it is not improbable that cells would
settle out of the slow-moving streams. Moseley (1871), while studying
the circulation in the wings of Blatta orientalis, noted that “the corpuscles
attach themselves to the inner wall of the vessel.” Yeager and Hendrick-
son (1934), who refer to the preexisting literature on the subject, have
recently observed the flow of blood in the wing veins of the roach Peri-
planeta americana L., and have noted that a portion of the blood cells in
these channels may, at times, temporarily cease to flow. Likewise, Vial-
lanes (1882) noted a similar phenomenon in the circulating blood of a
transparent fly larva. In their paper, Yeager and Hendrickson suggest
that the temporary cessation of corpuscular flow, especially if extended
to other blood channels of the body, would tend to produce a variation
in total blood cell count, provided the percentage of such non-circulating
cells is of sufficient variability. It is not improbable that this factor may
have affected these cricket counts. (2) It is not impossible that differ-
ences in the state of nutrition of individual insects (the amount of water
ingested, contained in, or given out from the animal’s body) may also
have affected the total count by causing a dilution or a concentration of
the circulating blood. Haber (1926) reports that if specimens of Blattella
germanica L. are fed dry food in a dry habitat the animals soon reach a
state in which it becomes difficult to obtain blood from them; well fed
individuals, on the other hand, contain more blood and bleed easily.
Moseley (1871) observed that if specimens of Blatta orientalis were de-
prived of food and water for several days, circulation in the insect would
become very feeble or almost absent. Muttkowski (1923), working with
Leptinotarsa larvae, found that the animals appeared turgid after feed-
ing due to the increased volume of blood plasma which distended the
hemocoel. If starved, the same larvae become flaccid and wrinkled due
to the decrease of blood plasma in the body. Berlese (1901), in discuss-
ing insect metabolism, pointed out the relationship which exists between
the digesting food in the alimentary canal and the plasma surrounding
it and noted the changes in the plasma as the digested material entered
the blood. The entrance of this food material undoubtedly would alter
the volume of blood in the body of the animal. Wigglesworth (1931, p.
OSCAR E. TAUBER AND J. FRANKLIN YEAGER

425) noted an increased amount of hemolymph in the bug, *Rhodnius prolixus*, following the ingestion of rabbit blood. Miall and Denny (1886), in discussing the blood of *P. orientalis*, state (p. 142), "The quantity varies greatly, according to the nutrition of the individual; after a few days' starvation, nearly all the blood is absorbed." Other workers, including Newport (1845) and Bruntz (1908), also stated that the blood volume of an insect may vary according to the nutritional condition of the individual. These variations in volume probably bring about changes in the concentration of corpuscles by concentrating or diluting the blood. (3) It has been shown that in the cricket (Yeager and Knight, 1933) as well as in the roach (Yeager, Shull, and Farrar, 1932) blood coagulation involves the formation of a cell coagulum. It is not impossible that cell coagulation, at the point of sampling (antenna), may account partially for the wide range of total count values for the cricket, although the authors believe such an effect to be negligible or very slight, since preparations were discarded whenever signs of cell coagulation appeared in the diluting pipette or the counting chamber. Furthermore, figure 4 shows that counts from normal animals are slightly higher than those from acid vapor treated animals whose blood was not in a coagulable state. Nevertheless, the very low counts given in the literature by Hardy (1892) for Astacus blood, and by Haber (1926) for *Blattella (Periplaneta) germanica* blood may well have been due in part to loss of cells by coagulation since these authors apparently used no effective anti-coagulant measures.

**SUMMARY AND CONCLUSIONS**

(1) Two hundred and twenty total blood (hemolymph) cell counts of the field cricket, *Gryllus assimilis pennsylvanicus* Burm, have been obtained by a slight (micro) modification of the usual hemacytometer technique.

(2) The entire series (220 counts) has a trimodal frequency distribution, skewed toward the higher counts (class intervals of 10,000 cells per mm$^3$ of blood).

(3) The skewness and trimodality of the entire series apparently are not due to maleness, to femaleness, to general growth (that is, difference between nymph and imago), to the month during which the animals were collected, to the existence of two broods per annum that overwinter, respectively, in the egg and nymphal stages, or to the anticoagulant action of glacial acetic acid vapor.

(4) It is suggested that the skewness may result, at least partially, from the occurrence of excessively high total blood cell counts accompanying certain physiological and pathological conditions such as ecdysis, oviposition, and parasitism; and possibly, in part, to certain circulatory factors inherent in the animal. The counts of 145,000 cells per mm$^3$ and higher were obtained from female crickets (Fig. 2). These tend to accentuate the asymmetry of the entire distribution (Fig. 1, B).

(5) The total blood cell counts of this cricket are widely distributed, ranging from about 15,000 to approximately 275,000 cells per mm$^3$ of blood, with an average of 70,118 cells per mm$^3$. Certain circulatory and nutritional factors characteristic of the insects may be responsible, in part, for the wide range of counts.

(6) In general, the average count from imagos is higher than that from nymphs.
TOTAL BLOOD CELL COUNTS: CRICKET

(7) The average total blood cell counts from June-July crickets and from September-October-November crickets differ significantly; it is tentatively suggested that the difference may be due to the local existence of two seasonal broods within this species.

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Yeager, J. Franklin and Harry H. Knight

Yeager, J. Franklin and Oscar E. Tauber

Yeager, J. Franklin and George O. Hendrickson
NOTES ON THE BIOLOGY AND CONTROL OF NEOSCIARA OCELLARIS (COMSTOCK) (DIPTERA, SCIARIDAE)

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Formerly the root gnats were included in the Mycetophilidae as a subfamily, Sciarinae, but recently taxonomists have accorded them family rank. The harmful effects caused by the feeding of their larvae on the underground portions of various plants were mentioned by early American entomologists; however, these injuries did not attract much attention in greenhouses and mushroom cellars until more recently. Many of the species of Sciaridae feed on the healthy tissues of higher plants, while, so far as is known, the Mycetophilidae feed exclusively on fungi or decaying organic matter. The primary purpose of these investigations was to study the biology of one species, Neosciara ocellaris (Comstock), common in greenhouses about Ames, Iowa. During the investigations it was found that the maggots were causing considerable damage to greenhouse plants, and thus some control measures were undertaken.

Neosciara ocellaris was found to be abundant in greenhouses at Ames, Iowa. Specimens have also been collected at Washington, D. C., and Lancaster and Buffalo, New York. It is not unlikely that the species occurs in every state, because of the ease with which the immature stages could be carried in the soil about the roots of nursery plants.

HISTORY AND IMPORTANCE

Walsh (1868) seems to have been the first writer to note that maggots of some species of sciarids were associated with a form of potato scab. In 1894 Hopkins made similar observations, and assigned to the insect which caused the damage the scientific name, Epidapus scabiei; however, the fly is now placed in the genus Pnyxia. Chittenden (1901) reported that maggots of Sciara were causing considerable damage to roots of potted plants, lettuce, cucumbers, and carnations, and Johannsen (1912) also found the maggots damaging roots of wheat, corn, potatoes, and other plants. In 1916 Hungerford published his studies of the biology of Neosciara coprophila (Lintner). He found the maggots of this species to be causing considerable damage to plants in conservatory windows and to potted plants. Gui (1933) reported that maggots of Pnyxia scabiei caused injury to potatoes in several localities in Ohio during the years 1926 to date (1933).

As far as the writer is aware, Neosciara ocellaris has not been mentioned in the literature as an injurious species, but it is not unlikely that it has many times been confused with Neosciara coprophila, a species

The writer is indebted to Dr. H. M. Harris, Iowa State College, for his many suggestions and constructive criticisms during the progress of these studies.
The adult flies of *N. ocellaris* usually are not noticeably abundant in greenhouses in Iowa during the summer, but when cool weather appears they increase in numbers in the greenhouse benches, and sometimes the maggots can be turned out of the soil in squirming masses. Under favorable conditions these flies increase rapidly, and the maggots cause considerable damage which first becomes apparent when the plants lose their healthy, vigorous growth.

**LIFE HISTORY**

*Technique.* The rearing records were obtained under constant conditions at 25° C. and nearly 100 per cent relative humidity. The larvae were reared individually in stender dishes with close fitting tops, pea leaves being used as food. In order to maintain sufficient moisture, the bottom of each stender dish was covered with a moist disc of ordinary paper toweling; this light background also aided in locating the cast head capsule. Each larva was examined twice daily to determine the time of ecdysis, these observations being made about 8:00 A. M. and 4:00 P. M. The width of the head capsule and the approximate length of the body was determined after each ecdysis.

*Eggs.* The eggs are oval in shape, 0.25 mm. in length and 0.09 mm. in width; the cephalic ends are slightly broader than the posterior ends. When first deposited they are pale greenish-yellow in color, but they soon change to a pearly white. During the third day of incubation the head of the developing embryo begins to darken and shows at the cephalic end as a spot with indefinite outline. The head of the embryo becomes quite dark and conspicuous before hatching, and at this time the larva is active within the chorion. The eggs are laid in irregular clusters of 3 to 40. They are usually placed in crevices in the soil or just below the surface of the soil along the stems of plants. Under greenhouse conditions the eggs usually hatch in four to five days; however, the incubation period may be only three and one-half days when kept at 30° C. and nearly 100 per cent relative humidity. The minimum temperature to which the ova were exposed was 20° C. and the maximum was 34° C.; under the former conditions the ova hatched in seven days, but under the latter conditions the embryos failed to develop. The eggs will not hatch unless they are in contact with moisture, or under conditions where the relative humidity is nearly 100 per cent. The larva escapes from the egg by eating a small hole in the cephalic end of the chorion, and if food is available it begins to feed immediately.

*Larva.* The larva is characterized by a strongly sclerotized, shining black head. It is 12-segmented, footless, more or less cylindrical, slightly tapering from the middle towards both ends, soft, translucent, and of a whitish color. There are eight pairs of conspicuous spiracles located along the sides. Although the larval life was greatly lengthened as a consequence of starving and of subjecting them to other adverse conditions, the number of instars was always four.

As there are no pronounced external morphological changes during larval development, it is not necessary to give a separate description of the larva in the various stages. The width of the head capsule and the approximate length of the body of the larva in the various stadia are given in table 1.
TABLE 1. Results of observations on the width of the head capsule and the length of the body of N. ocellaris larvae

<table>
<thead>
<tr>
<th>Instar</th>
<th>Number of observations</th>
<th>Mean width head capsule mm.</th>
<th>Mean width body mm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>50</td>
<td>0.08</td>
<td>0.65</td>
</tr>
<tr>
<td>2nd</td>
<td>50</td>
<td>0.13</td>
<td>2.00</td>
</tr>
<tr>
<td>3rd</td>
<td>50</td>
<td>0.21</td>
<td>4.00</td>
</tr>
<tr>
<td>4th</td>
<td>50</td>
<td>0.30</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Although the head capsule of a first instar larva is dark and sclerotized, it is never so strongly sclerotized nor so shiny-black as that of the older larva. There is a distinct increase in the width of the head capsule after each ecdysis, but no increase between molts, so the width of the head is an excellent character to use in determining the various instars.

There is no observable period of quiescence before ecdysis, except at pupation time. At the first, second, and third ecdysis the head capsule and outer body cuticula separate immediately posterior to the head and the head capsule splits along the median ventral line, the larva then crawls out of the old body covering, but at the last ecdysis, the head capsule splits along the median dorsal line, the head capsule and outer covering of the body separate as mentioned before. The process of molting requires only a few minutes, and it occurs wherever the larva happens to be feeding. Immediately after ecdysis the head is translucent, as is the remainder of the body. However, it soon begins to show pigment, and within a period of two to four hours it is again strongly pigmented. Although larvae have the head sufficiently hardened to feed immediately after hatching, newly molted larvae cannot begin to feed until the head becomes sclerotized.

Twenty of the larvae reared in these experiments (Table 2) were placed on fresh pea leaves and kept at a constant temperature of 25°C, and approximately 100 per cent relative humidity. The maximum time from egg to adult under these conditions was 25 days, and the minimum time was 21 days, the average being 23 days. Thirty larvae (Table 3) were kept under the same temperature and humidity conditions, but they were placed on leaves that were previously allowed partly to decay. The maximum time from egg to adult in this group was 25 days and minimum 18 days; the average being 19 days. There was, therefore, a difference of 4 days in the length of the larval life when fed on this material at different stages of freshness.

The maggots of Neosciara ocellaris are practically omnivorous in their feeding habits. The writer has observed them feeding on the decaying bodies of pupae and adults of their own kind, and on the roots and underground stems of the following plants: geranium, nasturtium, pea, potato, corn, grass, wheat, rape, lettuce, cucumber, and carnation. They were found also in great numbers in decaying onion bulbs, but it is not likely that they attack the healthy tissues. Even in soil in which plants are not growing, the maggots will develop apparently normally if there is an abundance of manure or other organic matter. The maggots as a
rule begin to feed first on the root hairs, gradually working inward to the larger roots and even to the underground stems. The writer has examined peas in which Neosciara maggots had eaten the root hairs and smaller roots, stripped the outer cambium from the larger roots and had even made extensive tunnels into the underground stems. The injury to roots is often severe enough to cause a sudden wilting and death to seedling plants, a result quite similar to that caused by certain seedbed diseases such as damping-off, and it is not unlikely that the injury resulting from the two causes are sometimes confused.

### TABLE 2. Length of life stages of *N. ocellaris* when fed on fresh pea leaves*

<table>
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<tr>
<th>Individual number</th>
<th>Egg</th>
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<th>Third instar</th>
<th>Fourth instar</th>
<th>Pupal stage</th>
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*Stated in days and at 25° C.

The maggots from *Neosciara ocellaris* are very susceptible to drying, and when the soil in which they are feeding becomes dry they are soon inactive, and unless moisture is added, death invariably results. If several maggots are feeding in close vicinity of each other they congregate in a group, when the soil becomes dry, and secrete a mucilaginous substance that for a short period tends to prevent desiccation.

The larva discontinues feeding about a day before it pupates. At the time when feeding ceases the compound eyes of the developing fly show above as two dark pigmented areas immediately posterior to the larval head capsule. With the aid of a microscope it can be seen that the body now contains a large amount of conspicuous fat bodies. Immediately after the larva stops feeding it commences to construct a silken chamber in which it later is to pupate. As construction proceeds, the pupal chamber becomes shorter and thicker and when completed is approximately 3 mm. in length and 1 mm. in width. Soon after the pupal chamber is
completed, which usually requires two days, the larva becomes quiescent and normally pupates within a few hours.

Pupa. Female pupae average about 2 mm. in length; the male pupae are slightly smaller. The legs are folded against the breast and venter, and the antennae are bent around the compound eyes and extend between the wings and legs. There are six abdominal and two thoracic spiracles; the prothoracic spiracles being located above the wing base, immediately behind the antennae. The pupa is white immediately after pupation, but it gradually becomes darker, and before emergence it has acquired the color pattern of the adult. As is shown in tables 2 and 3, the 50 larvae reared, with the exception of two, emerged on the fourth day after pupation, thus indicating that the length of the pupal stage is quite constant under these conditions.

A few hours before the adult is to emerge, the pupa usually works its way to the surface of the soil. This is accomplished by squirming motions in which, no doubt, the free appendages play an important role. In heavily infested greenhouses many pupae and pupal skins can be found with their posterior ends fastened to the soil and their anterior ends projecting into the air at about a 60 degree angle. Immediately before emergence, the adults can be seen squirming vigorously about in the pupal skin, which suddenly develops a T-shaped rupture. The transverse anterior part of the T-shaped opening, as is characteristic in other Orthorrhapha, is between the head and thorax and the stem of the T extends down the mid-dorsal line for about one-fourth the length of the abdomen. It requires only a few moments for the adult to work its way through this small opening. Then it remains inactive for a short time, allowing the wings to unfold and the body to become hardened. The

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Fig. 1. Showing length of life stages of Neosciara ocellaris.

When decomposing food is used
When green food is used
TABLE 3. Length of life stages of *N. ocellaris* when fed on decomposing pea leaves*

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*Mean 4 3 2 1 5 4 4 19

*Stated in days and at 25° C.

abdomen of a recently emerged female is greatly distended by the large number of eggs it contains.

*Adult.* Length of dried male specimens 1.5 to 2 mm.; width from tip-to-tip of wings 3.75 mm. Head black, antennae dark brown, less than three-fourths length of body, composed of 12 segments plus 2 subsegments; tarsal joints yellowish brown; pronotum light yellowish-brown; mesonotum yellowish in the center and darker at the edges; scutellum dusky brown; metathorax dark brown, almost black; abdomen with caudal portion of the segments blackish, cephalic portions yellowish-brown; claspers lighter brown; halteres light brown at base, the knobs blackish. Tibiae and tarsi dusky brown; femors paler, coxae still paler. Wings grayish, R₁ and R₂ dark, prominent, M₁ and M₂ not prominent; petiole of M indistinct; costa extending more than one-half distance from R₂ to M₁; R₁ ending near apex of wing; base of R₂ distal of the midpoint between the humeral cross-vein and the tip of R₁; petiole of Cu short, less than one-half as long as basal section of M₁. Claspers on the
dorsal-mesal margin with 2 or 3 strong setae in addition to 5 or 6 finer, apical ones, hypopygium with no median ventral lobe at base.

Length of dried female specimens 2 mm. to 2.5 mm.; width from tip-to-tip of wings 4.5 mm. Coloration same as for male except abdomen may show more yellow between segments, especially in gravid females where the abdomen is greatly distended, thus exposing more of the inter-segmental area. Wing venation same as for male.

The captive adults used in these experiments were fed on a 10 per cent sucrose solution, on which they lived for about the same length of time as adults under more natural conditions, the life-span being about a week. Mating usually occurs soon after the flies have emerged; coition generally taking only a few minutes, but sometimes lasting five to ten minutes. The male approaches the female from behind, and with the claspers, which are opened and closed spasmodically, the end of the female abdomen is grasped and then the male turns over to face in the opposite direction to that of the female, thus bringing the genitalia into juxtaposition. The species is both polygamous and polyandrous.

Females sometimes begin to oviposit late in the same day in which they emerge, but normally oviposition occurs during the second and third day. Some females lay all of their eggs in one day, but usually deposition extends over a period of two or three days. Twenty-five recently emerged females were kept individually in containers and the number of eggs laid by each determined. The maximum number laid by an individual was 175 and the minimum 123; the average being 140.

HABITS

The writer has watched the adults in the greenhouse feeding on the ooze that is found around decaying manure or other decaying organic matter, and so far as he is able to determine, they do not take any other under natural conditions. As a consequence of the feebleness of flight and their minute size, the adults usually escape the notice of all except the careful observer. Their color, which closely resembles that of the soil, and their secluded habits tend to make them rather inconspicuous. The flies apparently avoid the direct rays of the sun, and are found most abundantly in the shaded parts of greenhouses. Soil that is high in decaying organic matter and moist and shaded, seems to be ideal for their activities. The flies are strictly diurnal and do not become active until about eight o'clock in the morning, at which time they can be seen crawling from beneath particles of soil, vegetation, and out of crevices. The adults are strong runners but very weak flyers, often resorting to running to escape danger. A characteristic action of the males is to run hurriedly over the surface of the soil with their wings and antennae vibrating continuously. Although the females are rather active, they are not nearly so active as the males.

UNISEXUAL PROGENIES

While conducting life history experiments with Neosciara ocellaris, the writer found that the progeny from a given female are preponderantly of one sex. As these flies are polygamous, one male can be mated to several females with the result that some of the females give rise to female
progeny and others to male progeny, thus it can be shown experimentally that the factor or factors determining sex of progeny is held by the females and not by the male. The following table of rearing records show that some females produce female progeny while others produce male progeny.

**TABLE 4. Sex progeny of individual females**

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A review of the literature disclosed that Metz (1925) had previously observed this peculiar phenomenon in *Neosciara similans*, and he has shown that this condition occurs in several other species of Neosciara. He showed that the genetic basis responsible for sex of progeny was inherited in a simple Mendelian fashion. The female-producing female breeds as if heterozygous, and the male-producing female breeds as if homozygous recessive, for the gene or gene complex responsible for sex of progeny. Furthermore, he showed that the sex of the individual, as distinguished from sex of progeny, appeared to be dependent on an ordinary XX-XY sex-chromosome mechanism, the sperms containing XY chromosomes, therefore, were sex determining. As a male, when mated to two or more females, might give rise to both sons and daughters, it was assumed that he produced X-bearing and Y-bearing sperms in equal numbers. Metz's experiments indicated that in the female there was an elimination or inactivation of some of the sperms, which led him to conclude that in the female-producing female, the eggs were fertilized only by X-bearing sperms, while in the male-producing female only the Y-bearing sperms functioned, thus leading him to believe that sex was determined by two factors or complexes; the one acting directly and the other indirectly. The sex of the individual fly then depends directly on the type of sperm fertilizing the egg, but the type which would so function depends on the zygotic constitution of the female producing the egg.

**CONTROL**

Nicotine sulphate (Black Leaf 40), mercuric chloride, mercurous chloride, naphthalene, and calcium cyanide were used in an effort to determine an effective means of destroying Neosciara maggots. Naphthalene and calcium cyanide effectively destroyed the maggots when scattered over the soil in which they were feeding, but these compounds were decidedly harmful to the plants. Mercuric chloride at a concentration of
one ounce to eight gallons of water and mercurous chloride at a concentra-
tion of three to five ounces to ten gallons of water proved quite ef-
fective when the soil was thoroughly drenched with these concentrations. Nicotine sulphate was not effective in controlling the maggots. Several writers have stated that Neosciara maggots, from soil drenched with nicotine solutions, would not develop functional reproductive organs and that the females, therefore, would die without ovipositing. Data obtained by the writer from experiments conducted with this point in view did not indicate that the reproductive organs were impaired by the application of nicotine solutions, as flies emerging from larvae that had been feeding in soil thoroughly drenched with nicotine sulphate solutions reproduced normally.

Although maggots can be destroyed in the soil by the application of mercuric chloride and mercurous chloride solutions, they can also be easily kept in check, wherever it is practical to do so, by allowing the soil to dry out occasionally. Some of the plats of peas used in these experiments were watered daily, while other plats were watered twice a week or whenever the peas began to show need of water. The peas that were watered daily became heavily infested with Neosciara maggots resulting in serious injury, while those that were not watered so often showed only slight infestation, and apparently the peas were not injured. Several plats of peas were watered from below, thus allowing the surface of the soil to remain dry, with the result that they did not become infested with the maggots. A dry surface is very unattractive to the ovipositing females and even when they do oviposit there the eggs will not hatch as they require contact moisture. Sand also makes an unattractive surface for the ovipositing females, and where valuable plants are being injured by Neosciara maggots they can be protected by covering the surface of the soil with about one-half inch of dry sand.

A small predatory mite was very abundant in greenhouses at Ames, Iowa, and became so numerous that it quite effectually reduced the flies. The mites are predacious on the eggs of Neosciara, and often may be seen clinging to the bodies of the adults.

SUMMARY

Neosciara maggots, when occurring in large numbers, are capable of causing a great deal of damage to plants. They are easily disseminated in soil around plants; therefore, are probably widely distributed.

Although *N. ocellaris* breeds throughout the year in greenhouses, it is more abundant there during the winter and spring; they breed out doors only during the warmer months of the year. The adults frequent moist, shady places, and the eggs will not hatch and the larvae will not develop unless they are in contact with moisture.

At 25° C. and a high relative humidity (nearly 100 per cent), the eggs hatch in four to five days. Under natural conditions, the eggs are laid in clusters consisting of three to 40 eggs; the egg clusters being placed in crevices of the soil or along the underground stems of plants.

The larva is distinguishable from most dipterous larvae by a strongly sclerotized, shining black head. The body is semi-transparent, thus making it possible to see quite distinctly the internal organs.
On decomposing food, the average lengths of the instars are as follows: First 3 days, second 2 days, third 1 day, fourth 5 days; but when feeding on fresh food the larval life is lengthened 4 days. The maggots tend to be omnivorous, feeding on a large number of greenhouse and potted plants as well as on decaying animal tissues. Injury to the roots results in a sudden wilting and death to seedling plants, a result quite similar to that caused by certain seed bed diseases. The larva constructs a silken cocoon in the soil and transforms to an exarate pupa. The pupal stage, at 25° C. is four to five days.

The adults under natural conditions apparently feed exclusively on the ooze from decomposing organic matter; however, captive adults feed readily on a 10 per cent sucrose solution.

Mating occurs soon after the adults emerge, and normally oviposition begins in about two days.

The maggots can be controlled in the soil by drenching the soil with a mercuric chloride solution at a concentration of one ounce to eight gallons of water, and with a mercurous chloride suspension at a concentration of three ounces to ten gallons of water. They can also be kept in check by allowing the soil to dry out occasionally.

Neosciara ocellaris, as well as other species of Neosciara, produce "unisexual" progenies, but there are occasional exceptions.

A small predatory mite was common on the eggs of N. ocellaris in greenhouses at Ames, Iowa, and it became so numerous that it quite effectually reduced the population of flies.

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Thermogenesis in Hay-Inhabiting Fungi

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Thermogenesis by certain fungi having been demonstrated by Gilman and Barron (7), Miehe (18) and Norman (19), the way was opened for a more extensive investigation of the phenomenon. The question of how widespread the phenomenon was among other fungi and the relationship of heat liberation to respiration and growth of the fungous thallus were among the problems that were presented by the earlier work. In order to point the investigations to a definite end a substrate (hay) in which heating occurred frequently in nature, was selected; a definite number of organisms (14) commonly found on this substrate were isolated and the relations between heat production, carbon dioxide generation and growth were observed. The results of these observations are reported. Since the literature has been well reviewed in the papers cited above, the interested reader is referred to those authors for the history of microbial thermogenesis.

MATERIALS AND METHODS

ISOLATION OF ORGANISMS

The fungi which were used in these investigations were from two sources; first, isolations made from alfalfa hay which had heated spontaneously to about 60° C. in an experimental storage mow (11), and second, similar isolations obtained from some good quality hay that had been thoroughly wetted with sterile distilled water and incubated at room temperature.

Mow III from which the isolations were made had an initial moisture content of 36.8 per cent, being one of several mows of alfalfa hay stored with various initial moisture contents, for the study of the relation of moisture content to the keeping qualities of the hay. When this mow was opened many yellowish green patches were noted upon the hay; these proved to be composed of fruiting heads of Aspergillus flavus Link. Several samples of the hay from various levels to represent the whole were placed immediately in sterile moist-chambers. Later these samples were divided into three portions, which were incubated at room temperature, at 30° C., and at 40° C., respectively.

3Taken from a thesis submitted to the faculty of the Graduate College, Iowa State College, in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

The writer takes this opportunity to express his sincere appreciation to the members of the staff of the Botany Department, Iowa State College, for many valuable helps and constructive criticism which have facilitated this investigation. He is especially indebted to Dr. J. C. Gilman, on whose suggestion the investigation was undertaken and under whose direction it was carried out.

The final preparation of this paper for publication became the responsibility of Doctor Joseph C. Gilman due to the untimely death of Dr. Harrison after he had taken his degree and before the present paper was entirely completed.
As conidial heads appeared during the incubation period, they were picked off with a sharp nichrome needle and streaked on hay infusion agar in petri dishes. These streaks were allowed to stand for a few days at room temperature and were examined frequently. The pure cultures were transferred to agar slants. In the case of mixed cultures, spore suspensions in sterile water blanks were prepared and streaks made of the spore suspension; pure colonies were rather easily obtained by this means. Only four species of fungi were represented: *Aspergillus flavus*, *A. fumigatus* Fres., *A. terreus* Thom, and *Rhizopus tritici* Saito.

From the above substrate only the thermoduric forms of fungi were obtained. However, each of these forms proved able not only to endure heat, but also to raise considerably, by its own metabolic activity, the temperature of certain substrates. In addition to the thermoduric forms, many other fungi normally inhabit alfalfa hay. In order to isolate the latter, some good quality hay was thoroughly wetted with sterile, distilled water and incubated at room temperature. As conidia of fungi appeared they were picked off and transferred to agar slants. In addition some of the hay was washed with sterile, distilled water and the washings plated. Many colonies of fungi and bacteria appeared on the plates. The fungi were transferred to agar slants and the bacteria discarded. The following species were obtained: *Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. niger* Van Tieghem, *A. clavatus* Desm., *Penicillium oxalicum* Currie and Thom, *P. humicolum* Oudemans, *Spicaria divaricata* (Thom) Gilman and Abbot, *Mucor abundans* Povah, *Rhizopus tritici*, *R. nigricans* Ehrenberg, *Cunninghamella elegans* Lendner, *Hormodendron nigrescens* Paine, and *Alternaria humicola* Oudemans.

This group of fungi represents the more common inhabitants that may be found on hay growing under the conditions existing at the time the isolations were made. It is not to be considered a complete flora of that substrate.

In subsequent experiments several of these forms were found to release heat in considerable quantities although unable to survive the higher temperatures.

**PREPARATION OF SUBSTRATE**

For identification and for the study of growth rates, Czapek's medium, prepared in the usual manner (24), was used in all cases. Alfalfa hay for the thermogenesis experiments was obtained from the Iowa State College dairy farm. This hay was selected because it was of good quality, and it had been chopped by machinery into short, rather uniform lengths convenient for manipulation. A quantity of approximately sixty grams of the hay was placed in a number of glass tubes, 18" x 11/4", which were plugged at each end with cotton, and sterilized at 15 pounds pressure for one hour on each of four consecutive days. This treatment, though severe, was found to be essential to assure complete sterilization. The moisture content was then determined. This determination was necessary in order that the proper adjustment could be made by the addition of sterile water at the time of inoculation.

**PREPARATION OF THE FLASKS**

Ordinary commercial thermos flasks, 40 cc., capacity, were used in all experiments. These flasks were first thoroughly washed in boiling water...
and then kept in a 1-500 solution of mercuric chloride for several days. Just before using, they were rinsed several times, first with scalding water from the high pressure steam boilers, and finally with several changes of cool sterile, distilled water. The latter helped to cool the flasks to room temperature. The flasks were closed with rubber stoppers which had been treated with 50 per cent alcohol. It was later found by Gaskill (6) that the flasks could be plugged with cotton and successfully sterilized in the autoclave.

PACKING THE FLASKS

To pack the flasks the plug was first removed from one end of the tube of hay, inverted over the mouth of a sterile flask; the hay was pushed ahead of the upper plug into the flask by means of a glass rod. The flask was immediately closed with a rubber stopper which had been treated with 50 per cent alcohol. The operation was carried on in the inoculating chamber and the usual precautions taken to prevent contamination.

INOCULUM AND INOCULATION

In the inoculation of the substrates, there were two problems to be considered: the even distribution of the spores and the proper adjustment of the moisture content. Large cultures of the desired organism were grown on agar slants in sixteen-ounce flat bottles. As soon as sufficient quantity of spores was produced, the slant was washed with enough sterile, distilled water to bring the contents of the flask to the desired moisture content. The spore suspension was poured into the hay and the flask was closed with either a sterile rubber stopper or a cotton plug, the stopper carrying a sterile thermometer. The inoculated flasks were then rolled for about fifteen minutes to even the distribution of the moisture and of the spores, after which they were allowed to lie on the side for an hour during which they were rolled at frequent intervals.

For control there was used a flask loaded with sterile hay, brought to the same moisture content as the inoculated flasks. In a few cases a dry control was also used, but as it made no contribution of useful information, it was dropped from subsequent experiments.

METHODS OF OBSERVATION

Method of Determining Growth Rates

Growth rates were determined on petri dishes containing 10 cc. of Czapek's medium streaked with three drops of a spore suspension of the organism to be observed. A sufficient number of plates was inoculated to allow one or more to be incubated at each interval of five degrees from 0° C. to 50° C.

As germination occurred, the developing hyphae were measured frequently with an eyepiece micrometer until the spore could no longer be seen. As a rule twenty developing hyphae were selected at random over a small area, and measured, and calculation made of their average length. To give some index of comparison, the number of microns of growth was divided by the number of hours and the quotient recorded as the growth index.
This program was carried on in convenient series until all the organisms had been grown at each temperature interval. Although it was necessary to divide the work into small sections to facilitate handling, the conditions of the experiment were kept as nearly constant as possible.

**Determination of Thermogenesis**

During the study of thermogenesis two objectives were sought: first, a measurement of the rise in temperature, and second, the relation of this rise to respiration. Therefore, two series of observations were made; one to determine temperature alone and another to obtain temperature readings simultaneously with the measurement of carbon dioxide production.

In the first series the flasks were closed with cotton plugs and allowed to stand on the laboratory tables, subject to the temperature changes of the room. Readings were made by means of thermometers, usually at 9:00 a.m. Record was made of each flask, of the control, and of the room temperature.

In the second series the flasks were so arranged that temperature readings could accompany the determination of carbon dioxide evolution. For this purpose a thermocouple was passed through the rubber stopper into the mass of heating material and the temperature read on the dial of a potentiometer graduated directly in degrees Fahrenheit. These readings were immediately converted into degrees Centigrade and recorded. At each reading the accuracy of the potentiometer was checked against a calibrated thermometer in warm water and the instrument adjusted as required. To facilitate handling a number of flasks, a rotary switch was placed between the flasks and the potentiometer.

**Determination of Carbon Dioxide Evolution**

To determine the rate of carbon dioxide evolution, special absorption towers (Fig. 1), a modification of those described by Emerson (5), were built. Immediately following the first daily temperature reading, the vitiated air was drawn from the flasks and passed through a n/10 solution of barium hydroxide. Aspiration was continued until the entire air of each flask had been displaced, as nearly as possible, by carbon dioxide-free air. After allowing sufficient time for the precipitate to settle out, the collection tubes were detached and the contents rapidly filtered through a tared filter paper. After drying, the precipitate was weighed, and the carbon dioxide solution in milligrams per hour was calculated. This method can be relative only, but should furnish an index of the relation between carbon dioxide and the rate of heat production.

*Observations were also made on several organisms with the flasks placed in the constant temperature bath. The purpose of the experiments carried on in the water bath was to determine the effect of the fluctuations of room temperature on the progress of the heat curve and on the maxima. The observed differences in temperature between a series of flasks held in the constant-temperature bath and a series held at room temperature were no greater than the differences between individual members of either of the series. For the present experiment, the water-bath was considered an unnecessary refinement.*
THERMOGENESIS IN HAY-INHABITING FUNGI

Fig. 1. A. The CO₂ absorption apparatus. 1. Shell filled with Ba(OH)₂. 2. Intake tube from flask. 3. Rubber to hold 5 in place. 4. Outlet from absorption shell. 5. Absorption shell made out of test tube. 6. Rubber connection with detachable tube. This connection may be closed by means of a pinch cock. 7. Detachable tube for collecting BaCO₃. 8. Rubber tube to facilitate refilling. 9. Tube connecting units in series, and the series with the suction pump.

B. Thermos flask with attachments. 1. Intake for CO₂ free air. 2. Outlet for vitiated air connected to A2. A. Copper–constantin junction. 4. Insulated wires to switch-board.

C. Trap containing strong KOH.
In order to carry out a logical presentation of the results, the data on growth have been separated from those on the temperature-carbon dioxide relations of the organisms concerned. These two sub-groups were further divided for convenience so as to bring the data collected from related organisms together. Three natural groups occur: the species of Aspergillus, the Mucorales, and the Hyphales, containing two species of Penicillium and one each of Spicaria, Hormodendron, and Alternaria.

The data presented in all cases are summaries of the readings made on several individual cultures or flasks. No carbon dioxide readings are recorded for any of the controls since the amount of carbon-dioxide given off by the control flasks was so small that it was not measurable by the means used in these experiments.

**GROWTH INDEX**

As explained under materials and methods, the growth index for each organism was obtained by measuring the actual growth increment in microns for a given time and dividing the number of microns obtained by the number of hours required to make the increase.

When the data on the growth indices of the five species of Aspergillus (Table 1) are examined, it is evident that except at the optimum temperature or above, the most active rate of growth occurred in the 25-84 hour period. In the case of *Aspergillus flavus* and *A. niger* the growth at the optimum 30° C. was greatest during the 9-24 hour period. With *A. terreus* and *A. clavatus* the growth rate during the second and third period was the same at the optimum temperature of 35° C. *Aspergillus fumigatus* had the highest optimum at 40° C. Its highest index, 64, occurred in the 25-48 hour period. Above the optimum, the growth rate of the third period, 25-48 hours, was frequently less than that of the second. A similar condition was found with the members of the Mucorales studied. (Table 2.) In *Mucor abundans* the optimum was apparently at 25° C., although high indices were found at 30, 35, and 40° C. Except at the last temperature the rate was greatest during the third period, reaching its maximum at 130 at 25° C. The optimum for *Rhizopus tritici* was also 25° C. during the third period with an index of 350. *Rhizopus nigricans* showed a lower optimum between 20 and 25° C., but the growth during the period 25-48 hours was so rapid that measurements were impossible since the hyphae had completely covered the surface of the culture dish.

In the case of the third group of fungi the greatest growth occurred in the third period of time except at 35° C. with *Penicillium oxalicum*, at 10, 25, and 30° C. with *Alternaria humicola* (Table 3), and at 10 and 15° C. with *Spicaria divaricata*. The optimum temperatures for the five organisms under the conditions used were: *Penicillium oxalicum*, 25° C.; *P. humicola*, 20° C.; *Spicaria divaricata*, 30° C.; *Hormodendron nigrescens*, 15° C.; and *Alternaria humicola*, 25° C. The indices follow the general trend of the previous groups; that is, when the temperature was such that there was markedly stimulated growth in the second period, there was usually a rapid fall in growth rate in the succeeding period.
TABLE 1. Growth indices for the species of *Aspergillus* as determined by μ/hr.

<table>
<thead>
<tr>
<th>Degrees C.</th>
<th>Aspergillus flavus</th>
<th>Aspergillus terreus</th>
<th>Aspergillus niger</th>
<th>Aspergillus fumigatus</th>
<th>Aspergillus clavatus</th>
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In general, each form studied increased its growth activity as temperature increased, until the optimum was reached, after which a sharp decrease in growth occurred. However, there were some apparent exceptions; for example, in *Penicillium oxalicum*. In the study of this form it was found that at 10°C the growth index was 18, at 15°C it was 71 and at 20°C, a very favorable growth temperature, the growth index was only 30. A closer examination of the experimental plates showed that at 15°C only 50 per cent of the spores had germinated, but at 20°C germination was 100 per cent. Increased germination resulted in crowding and inhibition of the growth of the individual hyphae. Microscopic measurement of the individual hyphae gave a greater growth index at 15°C than at 20°C, but there was no doubt that if the quantity of mycelium per spore seeded could have been accurately determined, it would have been much greater at 20°C. It was purely a case where the acceleration of increased temperature was neutralized by the inhibiting effects of crowding. Several forms, especially those which tolerated or preferred the higher temperatures, were able to grow over a wide temperature range. A good example was *Aspergillus fumigatus*, which had its optimum above 40°C, which was able to make growth at 50°C, and, if allowed sufficient time, could grow well at 10°C. The growth activity of *Cunninghamamella elegans* was very interesting. At all the temperature intervals from 10°C to 40°C rapid growth occurred. From 25°C to 40°C the petri dishes were completely filled with mycelium in 48 hours. Another form, *Spicaria divaricata*, grew over a wide range of temperature, though the amount of mycelium produced at any time was very small. Two other forms, *Hormodendron nigrescens* and *Penicillium humicola*, preferred temperatures below 20°C. In table 4 is shown the optimum temperature for each form studied as determined by the growth index method.

### TABLE 4. Growth optima of organisms studied

<table>
<thead>
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<th>15°C–20°C</th>
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<td><em>Penicillium oxalicum</em></td>
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<td>A. niger</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhizopus tritici</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mucor abundans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spicaria divaricata</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cunninghamamella elegans</td>
<td></td>
</tr>
</tbody>
</table>
In all the experiments in thermogenesis alfalfa hay was used as the substrate. The severe treatment of the hay in sterilization may be open to some criticism because of chemical changes brought about during the process. All hay used in the experiment was treated in exactly the same manner; therefore, benefits or handicaps were the same for each organism studied and would not seriously affect the results. It was obvious that no living tissue of the alfalfa survived.

From the results which have been obtained it was shown that certain fungi were able to raise the temperature of alfalfa hay after all factors, other than those involved in the vital processes of the fungi themselves, had been eliminated. All the forms studied were not equal in their power to release heat; in fact, the thermogenic ability of some was almost negligible under the conditions of the experiment. These non-thermogenic species were usually either very slow in growing or they preferred lower temperatures for their maximum activities. It is easy to believe that such forms produced heat but that it was liberated no faster than it was dissipated. Figure 13 shows graphically that very little heat was produced by *Spicaria divaricata*. While this form grew over a wide temperature range, it produced only a small amount of mycelium. Another form, *Hormodendron nigrescens*, as shown in table 3, had an optimum growth temperature of 15°C. The temperature of the flask inoculated with *H. nigrescens* was at no time more than five degrees above the control. (Fig. 14.) It is possible that rising temperatures inhibited growth, and that inhibition was reflected in the release of heat.

*Aspergillus flavus* was the most active heat producer on hay. When placed on any suitable medium it germinated quickly and grew very rapidly. This characteristic was reflected in the temperature produced. As may be seen in figure 2, the temperature of a flask inoculated with *A. flavus* started to rise in a very short time, and continued until the maximum of 40°C. was reached on the third to fifth day. After the maximum was reached there was a rapid decline to a few degrees above the inoculated control flask. The new level was held for several days if the flasks were not opened. Two other species of *Aspergillus* found to be definitely thermogenic were *A. terreus* (Fig. 3) and *A. niger* (Fig. 4). Both forms raised the temperature of their substrate to about 40°C. or 14°C. above the control. In the present experiment, *A. fumigatus* (Fig. 5) released comparatively little heat. This was disappointing in view of the findings of several other investigators, namely, Miehe (18), James, Rettger and Thom (15), Norman (19) and Gilman and Barron (7). *Aspergillus fumigatus* was found by these workers to be very active on several types of substrate. For example, the data of Gilman and Barron show a maximum of 53°C. when grown on oats and of 31.2°C. on wheat. These data indicate that the type of substrate affects the amount of heat released by fungi. Hay, perhaps, was not a good substrate for *A. fumigatus*, or it may have been that the initial temperature was too low for the maximum activity of the fungus.

It was very interesting to note that the two species of *Rhizopus* and one species of *Mucor* were thermogenic. *Rhizopus tritici* (Fig. 9) and

---

*Table 5.*

*Table 6.*
Fig. 2. Aspergillus flavus. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

Fig. 3. Aspergillus terreus. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
TABLE 5. Temperature and carbon dioxide production in 60-gram samples of alfalfa hay by species of Aspergillus

<table>
<thead>
<tr>
<th>Days</th>
<th>Aspergillus flavus</th>
<th>Aspergillus terreus</th>
<th>Aspergillus niger</th>
<th>Aspergillus fumigatus</th>
<th>Aspergillus clavatus</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp. degrees C.</td>
<td>CO₂ mg. per hr.</td>
<td>Temp. degrees C.</td>
<td>CO₂ mg. per hr.</td>
<td>Temp. degrees C.</td>
<td>CO₂ mg. per hr.</td>
</tr>
<tr>
<td>0</td>
<td>25.0</td>
<td>27.0</td>
<td>25.0</td>
<td>21.0</td>
<td>23.0</td>
<td>24.4</td>
</tr>
<tr>
<td>1</td>
<td>30.5</td>
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<td>27.8</td>
<td>22.3</td>
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<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>33.0</td>
<td>1.8</td>
<td>30.0</td>
<td>28.0</td>
<td>1.95</td>
<td>23.7</td>
</tr>
<tr>
<td>3</td>
<td>33.0</td>
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<td>35.0</td>
<td>31.0</td>
<td>2.1</td>
<td>25.6</td>
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<tr>
<td>4</td>
<td>38.5</td>
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<td>40.1</td>
<td>34.9</td>
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<td>5</td>
<td>40.0</td>
<td>2.6</td>
<td>40.0</td>
<td>34.0</td>
<td>2.25</td>
<td>24.7</td>
</tr>
<tr>
<td>6</td>
<td>37.0</td>
<td>3.1</td>
<td>37.0</td>
<td>29.0</td>
<td>2.9</td>
<td>25.3</td>
</tr>
<tr>
<td>7</td>
<td>36.5</td>
<td>2.8</td>
<td>33.0</td>
<td>27.0</td>
<td>2.25</td>
<td>26.9</td>
</tr>
<tr>
<td>8</td>
<td>33.0</td>
<td>2.1</td>
<td>30.0</td>
<td>26.5</td>
<td>2.25</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Readings taken at 9 a.m. daily. Each reading, including control, is composite of several observations. No carbon dioxide was found in controls.
### Table 6. Temperature and carbon dioxide production in 60-gram samples of hay by species of the Mucorales

<table>
<thead>
<tr>
<th>Days</th>
<th>Mucor abundans</th>
<th>Rhizopus nigricans</th>
<th>Rhizopus triticil</th>
<th>Cunninghamella elegans</th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temp. degrees C.</td>
<td>CO₂ mg. per hr.</td>
<td>Temp. degrees C.</td>
<td>CO₂ mg. per hr.</td>
<td>Temp. degrees C.</td>
</tr>
<tr>
<td>0</td>
<td>22.4</td>
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<tr>
<td>1</td>
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<td>1.2</td>
<td>23.0</td>
<td>1.8</td>
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</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>1.5</td>
<td>23.0</td>
<td>1.6</td>
<td>39.5</td>
</tr>
<tr>
<td>3</td>
<td>27.2</td>
<td>2.0</td>
<td>25.0</td>
<td>2.1</td>
<td>42.0</td>
</tr>
<tr>
<td>4</td>
<td>28.2</td>
<td>2.0</td>
<td>28.0</td>
<td>2.3</td>
<td>34.0</td>
</tr>
<tr>
<td>5</td>
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<td>30.5</td>
<td>2.2</td>
<td>30.5</td>
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<tr>
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<td>32.5</td>
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<tr>
<td>7</td>
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<td>31.5</td>
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<td>8</td>
<td>24.0</td>
<td>1.2</td>
<td>30.0</td>
<td>1.5</td>
<td>27.0</td>
</tr>
</tbody>
</table>
TABLE 7. Temperature and carbon dioxide production in 60-gram samples of hay by species of *Penicillium*, *Hormodendron* and *Alternaria*

<table>
<thead>
<tr>
<th></th>
<th><em>Penicillium</em></th>
<th></th>
<th><em>Spicaria</em></th>
<th></th>
<th><em>Hormodendron</em></th>
<th></th>
<th><em>Alternaria</em></th>
<th></th>
<th>Check</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>oxalicum</td>
<td>humicola</td>
<td>divaricata</td>
<td></td>
<td>nigrescens</td>
<td></td>
<td>humicola</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Temp.</td>
<td>CO₂ per</td>
<td>Temp.</td>
<td>CO₂ per</td>
<td>Temp.</td>
<td>CO₂ per</td>
<td>Temp.</td>
<td>CO₂ per</td>
<td>Temp.</td>
</tr>
<tr>
<td></td>
<td>degrees C.</td>
<td>hr.</td>
<td>degrees C.</td>
<td>hr.</td>
<td>degrees C.</td>
<td>hr.</td>
<td>degrees C.</td>
<td>hr.</td>
<td>degrees C.</td>
</tr>
<tr>
<td>Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>24.0</td>
<td></td>
<td>24.0</td>
<td></td>
<td>28.5</td>
<td></td>
<td>21.6</td>
<td></td>
<td>23.0</td>
</tr>
<tr>
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<td>0.4</td>
<td>25.5</td>
<td>0.8</td>
<td>28.5</td>
<td>0.3</td>
<td>24.3</td>
<td>0.7</td>
<td>23.0</td>
</tr>
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<td>29.5</td>
<td>0.6</td>
<td>25.0</td>
<td>0.7</td>
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<td>0.5</td>
<td>25.1</td>
<td>3.1</td>
<td>26.0</td>
</tr>
<tr>
<td>3</td>
<td>32.0</td>
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<tr>
<td>4</td>
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<td>1.0</td>
<td>22.0</td>
<td>1.4</td>
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<tr>
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<td>32.5</td>
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<td>0.7</td>
<td>27.0</td>
<td>0.3</td>
<td>22.8</td>
<td>1.0</td>
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</tr>
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<td>1.4</td>
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<td>0.1</td>
<td>23.5</td>
<td>1.6</td>
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</tr>
<tr>
<td>8</td>
<td>28.5</td>
<td>1.0</td>
<td>24.0</td>
<td>1.0</td>
<td>27.5</td>
<td>0.1</td>
<td>26.0</td>
<td>1.0</td>
<td>27.0</td>
</tr>
</tbody>
</table>

Each column, including check, is composite of readings of several observations. No carbon dioxide was found in the check.
R. nigricans (Fig. 10) raised the temperature of their substrates to 42.9°C and 32°C, respectively. *Mucor abundans* (Fig. 7) reached only 28.5°C, but this point was 6.5°C above the check flask. Miehe (18) reported 38°C for *R. nigricans* and over 50°C for *Mucor corymbifer*.

Another of the Phycomycetes, *Cunninghamella elegans*, was found to release exceptional quantities of heat. The series of experiments from which the data graphically recorded in figure 10 were obtained, showed a maximum temperature of 37°C on the third day, but in another observation over 40°C was obtained the second day. This form grew rapidly over a wide range of temperature and in addition it produced a large amount of mycelium.

Of the species of *Penicillium* studied, *P. oxalicum* (Fig. 11) was thermogenic while *P. humicola* (Fig. 12) was not. Studies on growth showed that *P. humicola* preferred a lower temperature for growth than did *P. oxalicum*. This difference in growth habit was reflected in the rapidity in which temperature was raised.

If the amount of heat released by fungi were studied on a quantitative basis by a series of experiments conducted with a calorimeter, the relative efficiency of each organism could, no doubt, be established.

A summarized list of the species studied for heat production with the maximum temperature attained, the difference between the maximum of the inoculated flask and the check, and the day on which the maximum was reached, for each form is given in table 8.

**TABLE 8. Maximum temperatures in degrees Centigrade developed by organisms grown on sterile alfalfa hay. Forty per cent moisture content**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Maximum Temp.</th>
<th>Temp. of control</th>
<th>Maximum temp. above control</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
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<td>44.4</td>
<td>25.0</td>
<td>19.4</td>
<td>3</td>
</tr>
<tr>
<td>A. terreus</td>
<td>41.0</td>
<td>27.0</td>
<td>14.0</td>
<td>4</td>
</tr>
<tr>
<td>A. niger</td>
<td>39.4</td>
<td>26.0</td>
<td>13.4</td>
<td>6</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>27.0</td>
<td>24.0</td>
<td>3.0</td>
<td>7</td>
</tr>
<tr>
<td>A. clavatus</td>
<td>28.6</td>
<td>24.0</td>
<td>4.6</td>
<td>6</td>
</tr>
<tr>
<td>Penicillium oxalicum</td>
<td>34.0</td>
<td>24.0</td>
<td>10.0</td>
<td>4</td>
</tr>
<tr>
<td>P. humicola</td>
<td>28.0</td>
<td>24.0</td>
<td>4.0</td>
<td>5</td>
</tr>
<tr>
<td>Spicaria divaricata</td>
<td>27.0</td>
<td>25.0</td>
<td>2.0</td>
<td>4</td>
</tr>
<tr>
<td>Mucor abundans</td>
<td>28.5</td>
<td>22.0</td>
<td>6.5</td>
<td>5</td>
</tr>
<tr>
<td>Rhizopus tritici</td>
<td>42.9</td>
<td>26.0</td>
<td>16.9</td>
<td>3</td>
</tr>
<tr>
<td>R. nigricans</td>
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<td>23.0</td>
<td>14.0</td>
<td>6</td>
</tr>
<tr>
<td>Cunninghamella elegans</td>
<td>40.0</td>
<td>28.0</td>
<td>14.0</td>
<td>3</td>
</tr>
<tr>
<td>Hormodendron nigrescens</td>
<td>25.5</td>
<td>20.5</td>
<td>5.0</td>
<td>4</td>
</tr>
<tr>
<td>Alternaria humicola</td>
<td>27.0</td>
<td>24.0</td>
<td>3.0</td>
<td>5</td>
</tr>
</tbody>
</table>

**Thermogenesis and Carbon Dioxide Production**

In an attempt to correlate thermogenesis with respiration in the fungi studied, the data showed in some cases a rather close similarity in the progress of the curve for carbon dioxide production to the curve of temperatures.

*Table 7.*
With Aspergillus flavus, A. terreus, Mucor abundans, Rhizopus tritici, and Penicillium oxalicum, the curves approximate each other rather well. In other cases, Aspergillus niger, A. fumigatus, A. clavatus, Rhizopus nigricans, Cunninghamella elegans, Penicillium humicol, Hormodendron nigrescens and Alternaria humicola, the carbon dioxide curve was indicative of much greater activity than was shown by the temperature recorded, and there was no apparent correlation of the two phenomena. With Spicaria divaricata the trends of the two curves were opposed. In no case could comparison of activity, unit of carbon dioxide for unit of heat, be made of different species of organisms even within closely related groups. An example of extreme divergence of the behavior of organisms is well illustrated by comparing the data for Aspergillus flavus (Table 4) with those of Alternaria humicola (Table 6). Here it is found
that *Alternaria humicola*, although it produced only enough heat to raise the substrate 3° C. above the check flask, produced a much greater quantity of carbon dioxide than was produced by *Aspergillus flavus*, which raised the temperature of its substrate almost 20° C. above the check flask. In figure 14 the curve for carbon dioxide production for *Hormodendron nigrescens* shows a very rapid rise to the second day followed by a rapid decline to the fifth day. From the evidence of these observations vital activity was more accurately measured by the amount of carbon dioxide produced than by the amount of heat released. Norman (19) obtained a closer relationship between carbon dioxide production and
heating, both with a mixed micro-flora and with a pure culture of Trichoderma sp. The factors influencing the relation between carbon dioxide and thermogenesis in the investigations here reported were not sufficiently controlled to allow of explanation of the discrepancy found.

When a substrate like hay was used, it was almost impossible to keep conditions for respiration constant, especially in closed vessels. Even with constant aeration, there was no guarantee against local matting of the
mycelium or caking of the substrate. If possible to section the mass all gradations from aerobic to anaerobic conditions would be found. Under anaerobic conditions degradation of carbohydrates would occur with the release of much smaller amounts of heat than under aerobic conditions.

**Thermogenesis and Growth**

Those forms of fungi found to be actively thermogenic were, in general, capable of active growth and produced large amounts of mycelium. When the growth indices were compared with the temperature produc-
tion it was found that the period of rapid accumulation of heat corresponded very closely to the period of rapid germination and growth on artificial media. This was well illustrated in the case of Cunninghamella elegans, in which the growth index for the 1 to 8 hour period was 5 and for the 9 to 24 hour period rose to 416 and continued too rapidly for measurement. Figure 10 shows a very rapid rise in temperature from 25°C to 40°C in 48 hours. The data indicate that thermogenesis is subordinate to the vital processes involved in growth, and heat results when more energy is released by the organism than is required for its growth.
Fig. 14. *Hormodendron nigrescens*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.

Fig. 15. *Alternaria humicola*. Relation of temperature to carbon dioxide production in sixty-gram samples of hay.
SUMMARY

Fourteen of the common hay-inhabiting fungi were isolated and studied. The growth response of each form to temperature was obtained on artificial media by measuring the growth increment of the hyphae and dividing by the number of hours required for the increase. This gave a growth index.

The growth optima obtained were: Hormodendron nigrescens and Penicillium humicola, 15° C. to 20° C.; Rhizopus nigricans, Penicillium oxalicum and Alternaria humicola, 25° C.; Aspergillus flavus, A. terreus, A. clavatus, A. niger, Rhizopus tritici, Mucor abundans, Spicaria divaricata and Cunninghamella elegans, 30° C. to 35° C.; Aspergillus fumigatus, 40° C.

Each organism was inoculated in pure culture on sterile alfalfa hay in thermos flasks, the substrate brought to 40 per cent moisture content and thermogenesis and carbon dioxide evolution measured. Each form was able to raise the temperature of its substrate to some degree. Aspergillus flavus, A. terreus, A. niger, Penicillium oxalicum, Rhizopus tritici, R. nigricans, and Cunninghamella elegans proved to be decidedly thermogenic. Mucor abundans and Hormodendron nigrescens were thermogenic to a lesser degree.

Under the conditions of the experiments, Aspergillus fumigatus, A. clavatus, Penicillium humicola, Spicaria divaricata and Alternaria humicola developed little or no heat.

Though the curve for carbon dioxide evolution was in many cases parallel to that for heating, carbon dioxide production could not be used as a measure of thermogenesis.

Periods of rapid accumulation of heat in the inoculated flasks corresponded to periods of active germination and growth of the same organisms on artificial media.

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(20) ———

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(23) ————, AND M. B. CHURCH

(24) WAKSMAN, S. A., AND E. B. FRED

(25) WEHMER, C.
SHORTENING THE REST PERIOD OF THE TUBERS OF THE JERUSALEM ARTICHOKE, HELIANTHUS TUBEROSUS L.¹

E. S. HABER

From the Vegetable Crops Subsection, Iowa Agricultural Experiment Station

Accepted for publication June 21, 1934

Considerable interest has developed in the Jerusalem artichoke, Helianthus Tuberosus L., as a crop plant since the tubers of the plant have been found to be a source of production for levulose. Although the plant is native to North America it has never been cultivated here to the extent that it has in Europe, where the tubers are used for human consumption as well as stock feed. If it is to be planted extensively, a number of problems with regard to storage, propagation and seed must be solved. The tubers have a definite rest period after digging before growth will start. Is the length of the rest period influenced by temperature? Can the dormant period be shortened by the use of chemicals? Does the maturity of the tuber affect the rest period and what effect does the maturity of the tuber have on germination? Some experiments are reported in this paper which attempt to answer the foregoing questions.

REVIEW OF LITERATURE

The literature on the rest period of the Jerusalem artichoke tuber is limited, although many reports have been made on the dormancy of potatoes, gladiolus, woody plants, seeds and bulbs. Boswell (2) used 145 varieties or strains of this plant to determine the range in the length of the rest period of the tubers. The time required for 50 per cent of the seed pieces to sprout ranged from 54 to 200 days for the various lots.

Since the Jerusalem artichoke plant produces a tuber, it would be expected that the reactions of the dormant tuber to treatment with chemicals would be similar to those of the tuber of the common potato, Solanum tuberosum. Denny (4, 5) found that the vapors of ethylene chlorohydrin were effective in causing sprouting of dormant potatoes. Thiourea also caused prompt sprouting and the development of more than one sprout per eye. A number of workers have employed chemicals of various kinds to break or shorten the rest period of potato tubers. Ethyl bromide was used by McCallum (15). Rosa (16, 17) reported favorable results with nitrate of soda and ethylene gas, although nitrate of soda was not always effective. Loomis (12) recommended storage temperature of approximately 30° C. for shortening the dormant period of potato tubers. Loomis and Evans (14) suggested that vegetative organs containing stored starch will have the rest period shortened by ethylene, ethylene chlorohydrin, ether and similar compounds, while parts of plants such as bulbs containing little starch will not show the same response.

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Denny and Stanton (10, 11) found ethylene dichlorid and ethylene chlorohydrin effective in breaking dormancy in several species of woody plants and discovered that dormancy is not systemic but localized in the buds only. Denny (6) treated freshly harvested gladiolus corms of several varieties with ethylene chlorohydrin but the results were variable, depending upon the stage of dormancy and the variety.

Traub et al (18) stored artichoke tubers successfully at 0 to 2° C. (32-35° F.) and a relative humidity of 89-92 per cent. Above 4.4° C. (40° F.) the tubers were found to be more susceptible to storage diseases and the tubers lost moisture rapidly and shriveled.

EXPERIMENTAL

In the fall of 1932 an experiment was under way to determine the effect of harvesting date on the yield of artichoke tubers. Ten hills were dug at weekly intervals, starting Sept. 17 and continuing until Oct. 31, when all the crop was harvested. It was found at that time that the hills produced about eight times as many tubers by weight when harvested on Oct. 31 as when harvested on Sept. 17. Tests made on the levulose content of the early harvested tubers showed them to yield less levulose than tubers harvested a month later. There immediately arose the question of the value of early harvested tubers for seed purposes. Will early harvested tubers or immature tubers germinate satisfactorily if used for propagation of the following crop? Tubers which had been dug at weekly intervals were stored in a concrete underground storage and plantings made weekly from each lot. Pieces of tubers containing at least one good eye and weighing from one to one and one-half ounces were planted in rich compost soil. Each of 20 pieces from each lot was planted in four-inch pots and placed in a greenhouse where the temperature ranged from 15 to 18° C. The variety used for this study was the Mammoth French White (Sibley strain). Of all the varieties grown on the station grounds this seems to be the best adapted as measured by yields of tubers.

The initial temperature of the storage room where the tubers were stored was about 70° C. There was a gradual decrease in temperature from Oct. 1 to Nov. 1, at which time the storage reached a temperature of about 2° C. Although this was a storage cellar without artificial refrigeration the temperature could be held remarkably constant, with fluctuations averaging about two degrees after Nov. 1.

Table 1 presents the percentages of germination secured from tubers dug at regular intervals and stored for various intervals. The observations of the percentage of germination were made on March 24, at least three months after the last planting of tubers. Many of the tubers which had not germinated by that time had decayed.

Tubers dug on Sept. 27 when immature were viable if stored at a cool temperature for sufficient length of time. Tubers stored at least 35 days after digging germinated 100 per cent. Tubers dug at that time but stored a shorter time before planting failed to germinate. Tubers dug on Oct. 3 germinated 80 per cent with a week shorter storage period, that is, when stored only 28 days before planting. The later the harvest date, the shorter the storage period necessary before planting to secure 100 per cent germination. This does not take into account, however,
that germination was hastened where the storage period was longer at lower temperatures.

Tubers stored until Nov. 21 before planting germinated much more promptly than tubers stored for shorter periods, even though the tubers were of the same maturity when dug. Since the length of storage and the temperature of the storage influenced the speed of germination, an experiment was planned to determine the effect of storage temperature on the rest period and rate of germination.

**TABLE 1. Effect of maturity and length of storage period on the germination of artichoke tubers**

<table>
<thead>
<tr>
<th>Date of harvest</th>
<th>Length of storage-days</th>
<th>Date planted</th>
<th>Percent-age germination</th>
<th>Date of harvest</th>
<th>Length of storage-days</th>
<th>Date planted</th>
<th>Percent-age germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 27</td>
<td>7</td>
<td>Oct. 3</td>
<td>0</td>
<td>Oct. 17</td>
<td>7</td>
<td>Oct. 24</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>&quot;</td>
<td>10</td>
<td>&quot;</td>
<td>14</td>
<td>&quot;</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>&quot;</td>
<td>17</td>
<td>&quot;</td>
<td>21</td>
<td>Nov. 7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>&quot;</td>
<td>24</td>
<td>&quot;</td>
<td>35</td>
<td>Nov. 21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>&quot;</td>
<td>31</td>
<td>100</td>
<td>&quot;</td>
<td>Nov. 24</td>
<td>100</td>
</tr>
<tr>
<td>Oct. 3</td>
<td>7</td>
<td>&quot;</td>
<td>10</td>
<td>Oct. 24</td>
<td>7</td>
<td>Oct. 31</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>&quot;</td>
<td>17</td>
<td>&quot;</td>
<td>14</td>
<td>Nov. 7</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>&quot;</td>
<td>24</td>
<td>20</td>
<td>&quot;</td>
<td>Nov. 21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>&quot;</td>
<td>31</td>
<td>80</td>
<td>&quot;</td>
<td>Dec. 21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>Nov. 7</td>
<td>60</td>
<td>&quot;</td>
<td>58</td>
<td>Nov. 24</td>
<td>100</td>
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<tr>
<td>Oct. 10</td>
<td>7</td>
<td>Oct. 17</td>
<td>0</td>
<td>Oct. 31</td>
<td>7</td>
<td>Nov. 7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>&quot;</td>
<td>24</td>
<td>80</td>
<td>&quot;</td>
<td>Nov. 7</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>&quot;</td>
<td>31</td>
<td>100</td>
<td>&quot;</td>
<td>Nov. 7</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>Nov. 7</td>
<td>100</td>
<td>&quot;</td>
<td>51</td>
<td>Dec. 21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>&quot;</td>
<td>21</td>
<td>80</td>
<td>&quot;</td>
<td>Dec. 21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Dec. 21</td>
<td>100</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Tubers were dug on Oct. 30, 1933, and placed in the following storage temperatures: -2 C., -0.5 C., 2 C., 4.5° C., 10° C. and 30° C. Since the periderm of the artichoke is very thin and there is a rapid loss of moisture at higher temperatures, it was necessary to store the tubers at the higher temperatures in dry sand to prevent excessive shriveling. All tubers at all storage temperatures were stored in dry sand. At semi-monthly intervals—the first and sixteenth of each month—tubers were removed from each storage, cut into one to one and one-half ounce pieces with at least two eyes and planted in four-inch pots. The pots were placed in a greenhouse at a temperature of about 15° C. The temperature fluctuated about three degrees in either direction. Tubers stored at -2° C. were held for 24 hours at 2° C. in order to thaw slowly. Records were kept on the date of the appearance of sprouts above ground. The results are presented in tables 2 to 8, inclusive.

In the tables, the number of days required before sprouts appeared above ground are expressed in columns headed 25, 50 and 75 per cent sprouting. The appearance of the first sprout is not a good index of the length of the rest period, because with certain treatments one tuber may
germinate but considerable time elapse before the germination of another. Decay of tubers or the failure to secure 100 per cent sprouting because of bud injury makes it undesirable to use complete germination as a comparative index.

**TABLE 2. Tubers stored at seven different storage temperatures from Nov. 1 to Dec. 1**

<table>
<thead>
<tr>
<th>Storage temperature °C.</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
</tr>
<tr>
<td>-2.0</td>
<td>58 days</td>
<td>65 days</td>
</tr>
<tr>
<td>-0.5</td>
<td>68</td>
<td>74</td>
</tr>
<tr>
<td>2.0</td>
<td>64</td>
<td>77</td>
</tr>
<tr>
<td>4.5</td>
<td>74</td>
<td>79</td>
</tr>
<tr>
<td>10.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>24.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
<tr>
<td>30.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
</tbody>
</table>

**TABLE 3. Tubers stored at six different storage temperatures from Nov. 1 to Dec. 15**

<table>
<thead>
<tr>
<th>Storage temperatures °C.</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
<th>Ave. ht. stalks Feb. 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
<td>75 per cent</td>
</tr>
<tr>
<td>-2.0</td>
<td>19 days</td>
<td>28 days</td>
<td>40 days</td>
</tr>
<tr>
<td>-0.5</td>
<td>30</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>2.0</td>
<td>31</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>4.5</td>
<td>35</td>
<td>39</td>
<td>45</td>
</tr>
<tr>
<td>10.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Failed to sprout</td>
</tr>
<tr>
<td>24.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
</tr>
</tbody>
</table>

**TABLE 4. Tubers stored at six different storage temperatures from Nov. 1 to Jan. 1**

<table>
<thead>
<tr>
<th>Storage temperature °C.</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
<th>Ave. ht. stalks Feb. 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
<td>75 per cent</td>
</tr>
<tr>
<td>-2.0</td>
<td>14 days</td>
<td>18 days</td>
<td>21 days</td>
</tr>
<tr>
<td>-0.5</td>
<td>20</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>2.0</td>
<td>20</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>4.5</td>
<td>27</td>
<td>31</td>
<td>34</td>
</tr>
<tr>
<td>10.0</td>
<td>35</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>24.0</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Failed to sprout</td>
</tr>
</tbody>
</table>
Tubers that were stored at a temperature of 10° C. or above from time of harvesting (Nov. 1) to Dec. 15 failed to germinate. Most of the tubers had rotted four months after planting. Although Loomis (12) found that the rest period of potato tubers stored at 30° F. was shortened considerably, this temperature did not shorten the dormant period of the artichoke tubers. Tubers stored at this temperature failed to germinate when planted. It was impossible to store the tubers at this temperature for more than two weeks, even when the tubers were covered with sand, as excessive shriveling occurred. The lower the temperature at which the tubers were stored, the shorter the rest period until freezing temperatures were reached. Slight freezing hastened germination but heavy freezing was injurious to the tubers. Tubers

<table>
<thead>
<tr>
<th>Storage temperature °C.</th>
<th>No. days from planting to sprouting 25 per cent</th>
<th>No. days from planting to sprouting 50 per cent</th>
<th>No. days from planting to sprouting 75 per cent</th>
<th>Ave. no. of sprouts per seed piece Mar. 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.0</td>
<td>13 days</td>
<td>15 days</td>
<td>16 days</td>
<td>1.6</td>
</tr>
<tr>
<td>-0.5</td>
<td>17 &quot;</td>
<td>20 &quot;</td>
<td>21 &quot;</td>
<td>1.5</td>
</tr>
<tr>
<td>2.0</td>
<td>16 &quot;</td>
<td>20 &quot;</td>
<td>20 &quot;</td>
<td>1.2</td>
</tr>
<tr>
<td>4.5</td>
<td>16 &quot;</td>
<td>20 &quot;</td>
<td>21 &quot;</td>
<td>1.1</td>
</tr>
<tr>
<td>10.0</td>
<td>22 &quot;</td>
<td>26 &quot;</td>
<td>28 &quot;</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage temperature °C.</th>
<th>No. days from planting to sprouting 25 per cent</th>
<th>No. days from planting to sprouting 50 per cent</th>
<th>No. days from planting to sprouting 75 per cent</th>
<th>Ave. no. of sprouts per seed piece Mar. 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.0</td>
<td>12 days</td>
<td>12 days</td>
<td>13 days</td>
<td>1.6</td>
</tr>
<tr>
<td>-0.5</td>
<td>15 &quot;</td>
<td>16 &quot;</td>
<td>17 &quot;</td>
<td>1.6</td>
</tr>
<tr>
<td>2.0</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>15 &quot;</td>
<td>1.2</td>
</tr>
<tr>
<td>4.5</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>17 &quot;</td>
<td>1.3</td>
</tr>
<tr>
<td>10.0</td>
<td>16 &quot;</td>
<td>17 &quot;</td>
<td>19 &quot;</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Storage temperature °C.</th>
<th>No. days from planting to sprouting 25 per cent</th>
<th>No. days from planting to sprouting 50 per cent</th>
<th>No. days from planting to sprouting 75 per cent</th>
<th>Ave. no. of sprouts per seed piece Apr. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.0</td>
<td>11 days</td>
<td>13 days</td>
<td>14 days</td>
<td>2.0</td>
</tr>
<tr>
<td>-0.5</td>
<td>11 &quot;</td>
<td>12 &quot;</td>
<td>13 &quot;</td>
<td>1.8</td>
</tr>
<tr>
<td>2.0</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>15 &quot;</td>
<td>1.8</td>
</tr>
<tr>
<td>4.5</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>15 &quot;</td>
<td>1.5</td>
</tr>
<tr>
<td>10.0</td>
<td>15 &quot;</td>
<td>16 &quot;</td>
<td>17 &quot;</td>
<td>1.3</td>
</tr>
</tbody>
</table>
stored for only one month, at $-2^\circ$ C., required 58 days from planting for 25 per cent germination, but tubers stored for one and one-half months at the same temperature germinated 25 per cent in 19 days from planting, or just about one-third of the time. Tubers stored at $10^\circ$ C. for one month and then planted, failed to germinate four months after planting, moreover, most of the tubers rotted; but when the tubers were stored for two months at the same temperature, they germinated 25 per cent in 35 days.

**TABLE 8. Tubers stored at five different storage temperatures from Nov. 1 to March 1**

<table>
<thead>
<tr>
<th>Storage temperature $^\circ$C.</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
<th>Ave. ht. stalks Apr. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
<td>75 per cent</td>
</tr>
<tr>
<td>$-2.0$</td>
<td>12 days</td>
<td>14 days</td>
<td>15 days</td>
</tr>
<tr>
<td>$-0.5$</td>
<td>12 ''</td>
<td>13 ''</td>
<td>14 ''</td>
</tr>
<tr>
<td>$2.0$</td>
<td>13 ''</td>
<td>14 ''</td>
<td>15 ''</td>
</tr>
<tr>
<td>$4.5$</td>
<td>13 ''</td>
<td>14 ''</td>
<td>15 ''</td>
</tr>
<tr>
<td>$10.0$</td>
<td>13 ''</td>
<td>14 ''</td>
<td>15 ''</td>
</tr>
</tbody>
</table>

A record was kept of the average number of sprouts per seed piece which developed. Tubers stored at the lower temperatures ($-2^\circ$ C. and $-0.5^\circ$ C.) averaged a greater number of sprouts after two and one-half months in storage than tubers held at the higher temperatures. The tendency to develop a greater number of sprouts per seed piece increased at all storage temperatures as the storage period was prolonged and the number increased inversely with the temperature, that is, the lower the temperature and the longer the storage period, the greater the number of sprouts per seed piece.

Records were taken of the height of each individual plant about a month after germination. Tubers stored three and one-half months at $-2^\circ$ C. before planting, germinated on an average of four days before tubers stored at $10^\circ$ C., but the plants grown from tubers stored at the higher temperature were taller one and one-half months after planting than those grown from tubers stored at the lower temperature.

In tables 9 to 13, inclusive, are presented the results of treating tubers with thiourea and ethylene chlorohydrin. When tubers were stored for one month at $4.5^\circ$ C. and then immersed for one hour and two hours in various percentages of thiourea in water, germination was accelerated. Soaking the tubers in 4 and 6 per cent solutions for one hour cut the time required for germination in half. Soaking in 2 per cent solution for two hours was as effective as 4 and 6 per cent for one hour. Soaking tubers in a 10 per cent solution for either one or two hours caused a delay of about five days in germination. The above figures refer to germination of 25 per cent of the tubers.

Tubers stored at $2^\circ$ C. for three and one-half months and then immersed in 2, 4 and 10 per cent solutions of thiourea for one and two hours did not germinate more quickly than check tubers not treated but stored at the same temperature. However, the tubers immersed in
4 and 10 per cent solutions produced more sprouts per eye than check tubers. Tubers immersed in 2 per cent solution did not produce any more sprouts than the check tubers. The average height of stalks one month after germination was higher with the check tubers than with the treated tubers, and tubers treated with the higher concentrations grew slower than tubers soaked in the solutions of lower concentration.

Table 9. Tubers soaked in thiourea after one month in storage at 4.5° C. Planted Dec. 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
</tr>
<tr>
<td>2 per cent—1 hr.</td>
<td>33 days</td>
<td>36 days</td>
</tr>
<tr>
<td>4 &quot; &quot; —1 &quot;</td>
<td>28 &quot;</td>
<td>30 &quot;</td>
</tr>
<tr>
<td>6 &quot; &quot; —1 &quot;</td>
<td>28 &quot;</td>
<td>30 &quot;</td>
</tr>
<tr>
<td>10 &quot; &quot; —1 &quot;</td>
<td>33 &quot;</td>
<td>37 &quot;</td>
</tr>
<tr>
<td>2 &quot; &quot; —2 hrs.</td>
<td>28 &quot;</td>
<td>31 &quot;</td>
</tr>
<tr>
<td>4 &quot; &quot; —2 &quot;</td>
<td>28 &quot;</td>
<td>28 &quot;</td>
</tr>
<tr>
<td>6 &quot; &quot; —2 &quot;</td>
<td>33 &quot;</td>
<td>37 &quot;</td>
</tr>
<tr>
<td>10 &quot; &quot; —2 &quot;</td>
<td>56 &quot;</td>
<td>70 &quot;</td>
</tr>
<tr>
<td>Check</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 10. Tubers soaked in thiourea after two and one-half months in storage at 2° C. Planted Feb. 15

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
<th>Ave. ht. stalks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
<td>75 per cent</td>
</tr>
<tr>
<td>2 per cent—1 hr.</td>
<td>12 days</td>
<td>12 days</td>
<td>14 days</td>
</tr>
<tr>
<td>4 &quot; &quot; —1 &quot;</td>
<td>14 &quot;</td>
<td>16 &quot;</td>
<td>18 &quot;</td>
</tr>
<tr>
<td>10 &quot; &quot; —1 &quot;</td>
<td>17 &quot;</td>
<td>19 &quot;</td>
<td>21 &quot;</td>
</tr>
<tr>
<td>2 &quot; &quot; —2 hrs.</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>15 &quot;</td>
</tr>
<tr>
<td>4 &quot; &quot; —2 &quot;</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>17 &quot;</td>
</tr>
<tr>
<td>10 &quot; &quot; —2 &quot;</td>
<td>15 &quot;</td>
<td>16 &quot;</td>
<td>18 &quot;</td>
</tr>
<tr>
<td>Check</td>
<td>13 &quot;</td>
<td>14 &quot;</td>
<td>15 &quot;</td>
</tr>
</tbody>
</table>

Tubers were also immersed in solutions of ethylene chlorohydrin. Concentrations of 2, 4, 6 and 10 cc. per liter were used and the tubers soaked for one and two hours after storage for one month at 4.5° C. The results were similar to those secured with thiourea, although thiourea caused sprouting on an average five days earlier than ethylene chlorohydrin. To secure 25 per cent germination the strength of the solution did not seem to affect the rate of germination, but to secure 75 per cent germination 10 cc. per liter was more effective than weaker concentrations of the solution.

Another series of treatments was used on tubers stored for one month at 4.5° C. The tubers were cut into pieces ready for planting. The cut tubers were dipped in concentrations of 10, 15, 30, 45 and 60 cc. per liter of ethylene chlorohydrin. The seed pieces were placed in glass quart jars. The solution was then poured in until the jar was full and
# TABLE 11. Tubers stored one month at 4.5° C. and then treated with ethylene chlorohydrin. Planted Dec. 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
</tr>
<tr>
<td>2 cc. per liter—1 hr.</td>
<td>33 days</td>
<td>65 days</td>
</tr>
<tr>
<td>4 cc.</td>
<td>33</td>
<td>43</td>
</tr>
<tr>
<td>6 cc.</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>10 cc.</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>2 cc.</td>
<td>36</td>
<td>54</td>
</tr>
<tr>
<td>4 cc.</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>6 cc.</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>10 cc.</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>Check</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>10 cc. per liter—48 hrs.</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>15 cc.</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>30 cc.</td>
<td>18</td>
<td>23</td>
</tr>
<tr>
<td>45 cc.</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>60 cc.</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>5 cc.</td>
<td>41</td>
<td>70</td>
</tr>
<tr>
<td>10 cc.</td>
<td>27</td>
<td>54</td>
</tr>
<tr>
<td>15 cc.</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>20 cc.</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>30 cc.</td>
<td>19</td>
<td>34</td>
</tr>
</tbody>
</table>

# TABLE 12. Tubers stored at 2° C. for two months, then treated with ethylene chlorohydrin, stored again at 2° C. and planted at weekly intervals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days from planting to sprouting</th>
<th>Av. ht. stalks at one month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
</tr>
<tr>
<td>Treated—stored 1 week</td>
<td>19 days</td>
<td>20 days</td>
</tr>
<tr>
<td>Check</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Treated—stored 2 weeks</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>Check</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Treated—stored 3 weeks</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>Check</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Treated—stored 4 weeks</td>
<td>15</td>
<td>28</td>
</tr>
<tr>
<td>Check</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>Treated—stored 5 weeks</td>
<td>14</td>
<td>16</td>
</tr>
</tbody>
</table>

# TABLE 13. Tubers stored at 2° C. three months, then at 24° C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. days from planting to sprouting</th>
<th>Ave. no. of sprouts per seed piece</th>
<th>Ave. ht. stalks at one month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 per cent</td>
<td>50 per cent</td>
<td>75 per cent</td>
</tr>
<tr>
<td>2 weeks at 24° C.</td>
<td>13 days</td>
<td>15 days</td>
<td>16 days</td>
</tr>
<tr>
<td>Check</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>4 weeks at 24° C.</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Check</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
</tbody>
</table>
then poured off; the tubers were shaken to remove the excess liquid and the jar sealed for 48 hours and then the tubers were planted. A similar experiment was performed using 5, 10, 15, 20 and 30 cc. of ethylene chlorohydrin per liter and the tubers sealed in the jars for 72 hours before planting.

Tubers that had been soaked in a solution of 30 cc. per liter for 48 hours sprouted more promptly than tubers treated with other concentrations. Nearly 50 per cent of the tubers treated with 30 cc. per liter and sealed for 72 hours failed to germinate. None of the ethylene chlorohydrin treatments caused multiple sprouting.

Tubers stored at 2° C. for two months were treated with ethylene chlorohydrin, dipping them in a 30 cc. per liter solution and sealing for 48 hours as in the previous experiment. The tubers were washed with tap water after removal from the jars and stored again at 2° C. At weekly intervals 20 pieces were planted and 20 pieces stored at 2° C., but untreated. The treated tubers germinated more slowly than the check tubers and after five weeks of storage the treated tubers failed to germinate and rotted in the soil in less than a month after planting. The stalks of treated tubers grew at the same rate after two weeks’ storage, but tubers stored three and four weeks produced stalks that grew at about one-half the rate of growth of the check tubers. Tubers stored at 24° C. immediately after harvesting, failed to germinate, so an experiment was run to determine the effect of this temperature on germination where the rest period was about over. Tubers which had been stored at 2° C. for three months were placed at 24° C. for two and four weeks and then planted. This had no effect, apparently, on the rate of germination, growth rate of stalks, nor on the average number of sprouts per seed piece.

Sodium nitrate had no effect on shortening the rest period. Tubers were soaked for one and two hours in 2, 4, 6 and 10 per cent solutions. Rosa (16) found sodium nitrate somewhat effective on potatoes.

Dormant tubers were exposed to temperatures of -15° C. and -20° C. for two weeks, but the tubers failed to grow when planted, although the tubers were thawed slowly at 2° C. before planting.

Tubers stored for one month at 10° C. which had failed to grow when planted for two months were placed at a temperature of -2° C. These tubers were planted in four-inch pots. The potted tubers were exposed to -2° C. for one week. The tubers sprouted in two weeks after returning to the greenhouse.

DISCUSSION

Tubers harvested on Sept. 27 germinated as promptly as tubers harvested Nov. 1 if stored for the same length of time at the same temperature. The tubers dug at the earlier date were smaller than those dug later and no doubt less mature, but maturity apparently does not affect germination. Tubers dug on Nov. 1 were from plants that were quite green until the foliage was killed by heavy freezing two days earlier. In this latitude the tops of the plants do not die until killed by frost, so that all tubers are dug from immature plants. The plants do not bloom until late September or October, so that all tubers might be classified as immature.
Storage of the artichoke tubers slightly below the freezing point shortens the dormant period of the tubers. The factors which cause this are for the most part unknown. Periods of exposure just below the point of injury may cause stimulation of the tissue into renewed activity. Covel et al. (3) suggests, in the case of plants subjected to low temperature, weakening of the plant membranes and allowing greater rates of diffusion of enzymes or food material. Appleman (1) found that oxygen supply to the tissue was a critical factor in experiments with the potato. He was unable to correlate the rest period with enzyme changes.

According to Loomis (13), various resting plant organs have been found to pass through their normal rest period in minimum time at temperatures either 15° C. above or 15° C. below normal (20° C.). Successful treatments have been accompanied by similar chemical responses in the storage tissues, whether due to high or low temperatures or chemical treatments, and have been characterized by accumulations of available carbohydrates, particularly sucrose, in the treated tissues. However, Denny (8) points out that the increase in sugar and decrease in starch may be a result of breaking the dormancy and not the cause of it.

Denny, Miller and Guthrie (9) studied catalase, peroxidase and oxidoreductase activity in chemically treated potato tubers but make no claim that the changes in enzyme activity are to be looked upon as the cause of the growth of buds or as furnishing proof of the causes of the previous state of dormancy.

Thiourea solutions caused the growth of two or more buds from a single eye of the tubers. At each eye or bud of the tubers there are the rudiments of several buds. Usually only one bud will grow, the growth of the rest of the accessory buds being inhibited. If the sprout which starts growth first is removed, other buds may become active. Treating the tubers with thiourea solutions when the rest period is over, or nearly so, has more effect on multiple sprouting than treatment when the tubers are quite dormant. More sprouts per seed piece were produced on tubers stored at lower temperatures than at the higher temperatures studied. However, usually more than one produced a sprout, while thiourea caused multiple sprouting of a single eye. The growth of more than one sprout from a single eye results in spindly sprouts. Since thiourea had more effect on multiple sprouting when the rest period was nearly over than when the tubers were quite dormant, and since more sprouts per seed piece were produced on tubers stored at lower temperatures than at the higher temperatures, injury to the primary bud in the eye may be the cause of multiple sprouting. Ethylene chlorohydrin and thiourea treatments delayed germination when applied to tubers no longer dormant; this result also suggests injury to the eye or bud. Loomis and Evans (14) found that onions could be forced in the early part of the rest period by splitting combined with forced injections of tap water. Water soak treatments were not used in these experiments, but since the sodium nitrate soak treatments were not effective in shortening the rest period, it is probable that tap water would not be effective.

SUMMARY

Experiments are reported in which the rest period of dormant tubers of the Jerusalem artichoke was shortened by storage at temperatures near or slightly below freezing. The length of the rest period or dormant...
stage was directly dependent on storage temperature; the lower the temperature down to slightly below freezing, the shorter the rest period.

Ethylene chlorohydrin and thiourea were somewhat effective in shortening the rest period. Sodium thiocyanate was not so effective as ethylene chlorohydrin and thiourea while sodium nitrate was not effective at all.

Tubers harvested on Sept. 27 germinated as promptly, although considerably less mature than tubers harvested on Nov. 1.

Thiourea caused multiple sprouting, which was more pronounced when tubers were treated after dormancy had been broken.

Tubers held for three or more months at temperatures near the freezing point exhibited a tendency to produce more than one sprout per seed piece.

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1. **Appleman, C. O.**  

2. **Boswell, V. R.**  

3. **Coville, F. V.**  

4. **Denny, F. E.**  

5.  

6.  

7.  

8.  

9.  

10.  
    **Loomis, W. E.**  

14. AND M. M. EVANS

15. McCALLUM, W. B.

16. ROSA, J. T.


18. TRAUB, H. P., C. J. THOR, J. J. WILLAMAN AND R. OLIVER
NOTES ON THE BIOLOGY OF ONCOPELTUS FASCIATUS
(DALLAS)

FLOYD ANDRE

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Accepted for publication June 27, 1934

Several years ago the writer became interested in a large black and
red lygaeid Oncopeltus fasciatus (Dallas) (Pl. I, A), which occurs quite
frequently on milkweed in Iowa. Upon investigating the literature, it was
found that very little is recorded concerning the life and habits of this
insect under field, greenhouse or controlled conditions of temperature and
relative humidity. The ability of this insect to live and reproduce nor­
mally when feeding on dried milkweed seeds and supplied with water,
makes it a very desirable form to study throughout the year.

CLASSIFICATION, HISTORY AND DISTRIBUTION

Oncopeltus fasciatus (Dallas) belongs to the group of insects com­
monly called “true-bugs,” of the order Hemiptera. It is placed in the
family Lygaeidae, which is widely known as the “chinch-bug” family.
Although many publications mention this species, little information is
given in the literature concerning its life and habits. Collecting records
and field observations constitute the bulk of published material.

Strangely enough, the first description of Oncopeltus fasciatus to be
recorded in literature was, through a mistake in identification by Herrich-
Schaeffer (1842), supposedly a description of Fabricius’s Lygaeus aulicus.
Dallas, however, in 1852 recognized from Herrich-Schaeffer’s description
and figure a new form which he named Lygaeus fasciatus. In this same
notation, Dallas states that the species was represented in the British
Museum by specimens from the United States, Brazil, Colombia and
British Guiana. Numerous records, which have greatly widened its known
distribution, have since been recorded in the literature; and it may now
be considered one of the most widely distributed species of Hemiptera in
the Western Hemisphere.

FOOD

As the vernacular name “milkweed bug” implies, Oncopeltus fasci­
atus (Dallas) feeds on numerous species of milkweed plants. Eggs,
nymphs and adults were collected from the flowers and pods of common
milkweed, Asclepias syriaca L., nymphs in the third and fourth instars
from pods of the whorled milkweed, Asclepias verticillata L., and several
mating pairs from the showy milkweed, Asclepias speciosa Torr., during
the summer of 1932.

Both nymphs and adults will suck the juices from grasses in an effort
to secure moisture. When wheat or bluegrass was grown in the rearing

*The writer wishes to express his appreciation to Dr. Carl J. Drake for many help­
ful suggestions toward the completion of this study, and to Dr. H. M. Harris for his
valuable criticisms.

[73]
cages, the bugs grew to maturity on dried milkweed pods and seeds without taking any water other than that obtained from the plants. Neither the wheat nor the bluegrass in itself was a sufficient diet, because all efforts to rear the insects on grass alone failed.

METHODS

The experiments presented in this paper have been conducted under (1) constant conditions of temperature and relative humidity, (2) in an out-of-doors screened laboratory, and (3) under greenhouse conditions. Constant temperature machines (Brindley and Richardson, 1931) were used to obtain the desired temperatures, and the relative humidities were kept constant by using saturated solutions of certain inorganic salts. The humidity was maintained at approximately 70 per cent by keeping pans full of a saturated solution of sodium chloride placed directly in the air currents from the fan. This relative humidity was used in all the studies made except in determining the incubation period of eggs.

The eggs were obtained from adults confined in breeding cages, which were merely medium-sized lamp chimneys with a piece of cheese cloth fastened over each end by means of a rubber band. These bugs were fed on dried milkweed pods which had been collected in the fall before the seeds had escaped. The females laid their eggs in pieces of cotton placed in the cages for that purpose. At least once a day, or oftener when an experiment required an accurate record of the time the eggs were laid, the cotton was taken out and the eggs removed by pulling it apart, as the female generally inserted her eggs some distance into the cotton. The eggs were then put into other cages or stender dishes and these were placed in the desired conditions. Nymphs were reared in these cages or in stender dishes with ground glass tops. Circular pieces of paper toweling were placed in the bottom of the stender dishes and moisture was supplied daily.

In order to determine the length of time required for incubation, the eggs were placed in 10 mm. by 50 mm. shell vials. These were placed in 20 mm. by 80 mm. vials containing a saturated solution of the salt, giving the desired relative humidity and tightly corked. The vials were aerated once a day.

DESCRIPTIONS

EGG

Egg elongate-oval, more broadly rounded at cephalic end, nearly twice as long as broad, circular in cross-section, without distinct sculpturing or color markings on chorion; cephalic end with indication of circular lid or cap, the latter surrounded by 11 to 13 fairly prominent chorial processes, which are short, slightly bent inward, strongly constricted at their bases, and enlarged and somewhat rounded at their apices. Freshly deposited eggs chrome lemon-yellow, gradually changing to reddish, the red eyes and general form of the embryonic insect becoming quite distinctly visible beneath the chorion as incubation progresses. Size (average of 150 eggs): Length, 1.41 mm.; width, 0.63 mm.

NYMPHAL INSTARS

The spotted milkweed bug passes through five distinct nymal instars in its growth to the adult. Each of these instars is quite individual
BIOLOGY OF ONCOPELTUS FASCIATUS

and displays characters that make it easily differentiated. A short de-
scription of each instar follows.

First Instar. Elongate oval, slightly larger than egg. Widest across
middle portion of abdomen. Tapers gradually toward head. Posterior
portion of abdomen slightly rounded. Head triangular, obtusely rounded
in front. Antennae linear, finely pubescent, about three-fourths as long
as body, inserted laterally, four segmented, the proportional length of the
Prothorax one and one-half times as long as wide, anterior margin con-
cave, sides widening to posterior. Abdomen widest at segment IX, sides
slightly rounded. Rostrum four-segmented, reaching beyond posterior
end of abdomen. Legs finely pubescent, tarsi two-segmented. Legs, an-
tennae and rostrum pale smoky brown. Joints of antennae between seg-
ments and last half of distal segment lighter. Head and thorax much
darker, the latter with a rather broad median reddish-orange stripe.
Abdomen reddish-orange, more yellowish at base. Width across widest
portion of abdomen, 1.1 mm. Length, 3.1 mm.

Second Instar. Slightly
darker than first, joints between anten-
Width across widest portion of abdomen, 1.210 mm. Length, 3.502 mm.

Third Instar. In this instar the nymph begins to show wing-pads
which appear as swellings on the posterior margin on both sides of the
metathorax. A darker color is prevalent in this stage than is found in
Width across widest portion of abdomen, 1.362 mm. Length, 4.166 mm.

Fourth Instar. Mesothoracic wings appear in this stage and nearly
cover the metathoracic pair. Antennae, head, wings and legs are black.
Body and margins of wing-pads covered with fine pubescence. Two spots
or stink gland holes on the third and fourth abdominal segments are more
prominent than in any previous stage. Antennal segments in the propor-
tion 8:26:22:34. Width across widest portion of abdomen, 2.017 mm.
Length, 6.211 mm.

Fifth Instar. Elongate-oval, widest across middle of abdomen, taper-
ing gradually toward head; posterior portion of abdomen broadly rounded.
Head triangular, obtusely rounded in front, slightly rugose, with more or
less pronounced dorso-median ridge broadly rounded, extending from
apex to middle portion posterior to which is a slight curved transverse
depression. Antennae linear, finely pubescent, about half as long as body,
inserted laterally, four-segmented, in the proportion 12:38:32:46. Eyes
lateral, prominent. Prothorax almost twice as wide as long, widened pos-
teriorly, the anterior margin concave, the sides straight, gradually widen-
ing to posterior margin of third abdominal segment. Lateral margins of
wing-pads slightly rounded. Sides of pronotum and abdominal segments
slightly rounded. Rostrum four-segmented, reaching beyond posterior
margin of thorax. Legs finely pubescent, comparatively long and slender.
Antennae, legs, rostrum, upper portion of head, posterior margin of pro-
thurax, lateral spots on all of the abdominal segments and median dorsal
spot on the fifth, sixth, seventh, eighth and ninth abdominal segments,
and apex of ventral abdominal surface black. Remainder of body reddish-
orange. Length at the end of this instar varies from 9 to 11 mm. Width
across widest portion of abdomen, 3.44 mm.

Adult. Elongate-oval. Color black and red; cheeks, side margins of
pronotum, basal and apical thirds of elytra usually reddish, sometimes fading to reddish orange. Apex of scutellum, coxae and abdomen for the most part, reddish yellow. Legs and antennae shining black. Genital plates, spots near middle of third and fourth ventrals and front angles of each ventral at sides black. Pronotum declivent in front, deeply impressed at each side. Antennal segments in the proportion 15:45:38:65. Width across widest portion, 4.8 to 6.0 mm. Length, 13 to 18 mm.

TABLE 1. Table of measurements

<table>
<thead>
<tr>
<th>Stage of development</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width across eyes</td>
<td>.511</td>
<td>.688</td>
<td>.946</td>
<td>1.290</td>
<td>1.706</td>
<td>2.193</td>
</tr>
<tr>
<td>Antennal I</td>
<td>.129</td>
<td>.215</td>
<td>.258</td>
<td>.344</td>
<td>.516</td>
<td>.645</td>
</tr>
<tr>
<td>Antennal II</td>
<td>.308</td>
<td>.473</td>
<td>.795</td>
<td>1.118</td>
<td>1.634</td>
<td>1.935</td>
</tr>
<tr>
<td>Antennal III</td>
<td>.301</td>
<td>.430</td>
<td>.688</td>
<td>.946</td>
<td>1.376</td>
<td>1.634</td>
</tr>
<tr>
<td>Antennal IV</td>
<td>.516</td>
<td>.738</td>
<td>1.079</td>
<td>1.462</td>
<td>1.988</td>
<td>2.769</td>
</tr>
<tr>
<td>Total length of antenna</td>
<td>1.254</td>
<td>1.856</td>
<td>2.820</td>
<td>3.870</td>
<td>5.514</td>
<td>6.923</td>
</tr>
<tr>
<td>Width greatest</td>
<td>1.102</td>
<td>1.210</td>
<td>1.362</td>
<td>2.017</td>
<td>3.441</td>
<td>4.8-6.3</td>
</tr>
</tbody>
</table>

LIFE HISTORY AND HABITS

INCUBATION OF EGG

Certain definite macroscopic changes accompany the development of the egg. Within a comparatively short period of time, ranging from six to twelve hours—depending largely on the temperature—the incubating egg gradually darkens and turns reddish in color. At a constant temperature of 34.5° C, the egg changes to a reddish hue in 12 hours and the eye spots become visible in 24 hours; at 29.5° C, it turns reddish in 18 hours and eye spots show in 36; and at 24.5° C, reddening is noticeable in 36 hours and eye spots become evident in about 60 hours. Hatching takes place at the end of 72 hours at 34.5° C., in 96 hours at 29.5° C., and in 144 hours at 24.5° C.

A short time before hatching, the form of the embryonic insect can be seen through the translucent shell-membrane with the aid of a good lens. Faint striations mark the sutures separating the abdominal segments and the head and thorax of the embryo. Two deep red pigmented spots near one pole mark the location of the eyes. The chorion, or shell, is thin and easily broken.

The egg is very susceptible to the influence of external factors, especially temperature and moisture. High temperatures, within limits, accelerate development, whereas low temperatures retard incubation. (See table 2.) Eggs placed in dry sand immediately begin to desiccate and soon lose much of their moisture content, shrivel and fail to hatch. Conversely, an excess of water in the sand interferes with development and in numerous instances prevents hatching.
**TABLE 2. Incubation period of eggs**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Relative humidity</th>
<th>No. of eggs</th>
<th>Hatched Number</th>
<th>Percentage</th>
<th>Incubation period. Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.5° C.</td>
<td>75%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>24.5° C.</td>
<td>33%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>29.5° C.</td>
<td>75%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>29.5° C.</td>
<td>33%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>34.5° C.</td>
<td>75%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>34° C.</td>
<td>33%</td>
<td>30</td>
<td>20</td>
<td>66.6</td>
<td>3</td>
</tr>
<tr>
<td>38.0° C.</td>
<td>75%</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Room</td>
<td>75%</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Room</td>
<td>33%</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Outdoors</td>
<td>75%</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>Outdoors</td>
<td>33%</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

**HATCHING**

At the moment of hatching, the cephalic end of the eggshell is irregularly longitudinally ruptured by pressure on the shell membrane by the nymph. An examination of several hundred egg shells after incubation failed to show in a single case where the rupture had taken place in the form of the circular lid or cap as indistinctly outlined on the external surface of the chorion. From 10 to 25 minutes are required for the insect to escape from the shell. It is quite interesting to observe the hatching nymph slowly unfold its appendages, the order usually being antennae, first pair of legs, second pair of legs and third pair of legs. After a period of from 10 to 20 minutes, the newly hatched nymph begins to move slowly about and soon starts feeding.

**FEEDING HABITS OF THE NYMPHS UNDER CONTROLLED CONDITIONS**

Soon after hatching, the nymphs begin moving in search of food. Upon finding a milkweed pod, one would stop, raise the anterior part of its body, and then explore the surface with its beak. As soon as a favorable spot was found, the beak would be inserted. Then the nymph, after feeding for several minutes, would remove its beak, crawl off the pod and seek water.

For food, dried milkweed pods and their seed furnished the sole diet, and a vial of water plugged with a wad of cotton furnished the water. It was necessary to provide water to rear or keep the milkweed lygaeid alive on seeds or pods of the milkweed plant.

**HABITS OF NYMPHS IN THE FIELD**

During the summers of 1932 and 1933 the writer made frequent trips to various patches of milkweed in the vicinity of Ames, Ottumwa, New Sharon, Iowa City and a few other points to observe the habits of this
insect under natural conditions. Nymphs began to make their appearance in the field during the last week in July. They are somewhat gregarious in habit, and it was not uncommon to find large numbers feeding on a single milkweed pod. Unless approached with caution, the whole group would fall to the ground and not infrequently "play possum" for several minutes.

ECIDYSIS

A few hours before molting, usually within two or three, the nymph stops feeding and moving about and becomes very quiet. Gradually the body assumes a slightly swollen appearance, and a light colored longitudinal line appears along the median dorsal parts of the head and thorax. In a few minutes the body begins to contract spasmodically until the cuticula ruptures along this light line and the insect within may slowly work itself out of its old "skin." During the molting period the nymph is in a very helpless condition and at the mercy of other members of the same species or other insects with cannibalistic propensities. Molting takes place on the under side of milkweed leaves in the field, thus avoiding the direct rays of the sun.

LENGTH OF INSTARS

The length of a stadium depends primarily upon temperature, humidity and food conditions. Nymphs are active, and growth is greatly accelerated by high temperatures. In the accompanying tables 3 to 6 and figures 1 to 5, the effect of temperature upon the rate of development from the time the eggs are laid until the adult stage is reached is illustrated.

TABLE 3. Summary of life-history at constant temperature of 34.5° C. and relative humidity of 70 per cent

<table>
<thead>
<tr>
<th>Females (12 individuals)</th>
<th>Stage</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>1st</td>
</tr>
<tr>
<td></td>
<td>Average days</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td>Range in days</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Males (16 individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days....</td>
</tr>
<tr>
<td>Range in days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Males and Females (30 individuals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days.....</td>
</tr>
<tr>
<td>Range in days</td>
</tr>
</tbody>
</table>
TABLE 4. Summary of life-history at constant temperature of 29.5° C. and relative humidity of 70 per cent

<table>
<thead>
<tr>
<th>Females (30 individuals)</th>
<th>Stage</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>1st</td>
</tr>
<tr>
<td>Average days...</td>
<td>4.00</td>
<td>5.83</td>
</tr>
<tr>
<td>Range in days</td>
<td>—</td>
<td>5-7</td>
</tr>
</tbody>
</table>

| Males (40 individuals)    | Average days... | 4.00 | 5.90 | 6.07 | 6.02 | 6.00 | 8.35 | 36.34 | 34.53 | 70.87 |
|                           | Range in days   | —    | 5-7  | 5-8  | 6-7  | —    | 7-10 | 34-38 | 29-39 | 65-76 |

| Males and Females (70 individuals) | Average days... | 4.00 | 5.87 | 6.57 | 6.04 | 6.11 | 7.55 | 36.14 | 32.56 | 68.70 |
|                                  | Range in days   | —    | 5-7  | 5-8  | 5-8  | 4-8  | 5-10 | 33-38 | 22-39 | 56-76 |

TABLE 5. Summary of life-history at constant temperature of 24.5° C. and relative humidity of 70 per cent

<table>
<thead>
<tr>
<th>Females (17 individuals)</th>
<th>Stage</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
<td>1st</td>
</tr>
<tr>
<td>Average days...</td>
<td>6.00</td>
<td>7.00</td>
</tr>
<tr>
<td>Range in days</td>
<td>—</td>
<td>6-8</td>
</tr>
</tbody>
</table>

| Males (13 individuals)    | Average days... | 6.00 | 7.07 | 7.00 | 6.39 | 5.92 | 10.46 | 42.84 | 34.36 | 77.30 |
|                           | Range in days   | —    | 6-8  | —    | 6-7  | 5-7  | 9-13  | 41-45 | 26-39 | 70-80 |

| Males and Females (30 individuals) | Average days... | 6.00 | 7.00 | 7.03 | 6.40 | 6.23 | 10.73 | 43.39 | 32.34 | 75.73 |
|                                  | Range in days   | —    | 6-8  | 7-8  | 5-7  | 5-7  | 9-14  | 41-46 | 26-39 | 70-80 |
Fig. 1. Summary of life-history at 34.5° C. and 70 per cent relative humidity.

Fig. 2. Summary of life-history at 29.5° C. and 70 per cent relative humidity.

Fig. 3. Summary of life-history at 24.5° C. and 70 per cent relative humidity.
TABLE 6. Summary of life-history in out-of-doors, screened laboratory

Females (13 individuals)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Egg</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days</td>
<td>6.00</td>
<td>8.07</td>
<td>6.61</td>
<td>6.00</td>
<td>7.07</td>
<td>12.92</td>
<td>46.57</td>
</tr>
<tr>
<td>Range in days</td>
<td>—</td>
<td>7-9</td>
<td>5-7</td>
<td>—</td>
<td>6-10</td>
<td>10-15</td>
<td>39-48</td>
</tr>
</tbody>
</table>

Males (17 individuals)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Egg</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days</td>
<td>6.00</td>
<td>8.11</td>
<td>6.70</td>
<td>6.00</td>
<td>6.17</td>
<td>12.00</td>
<td>44.98</td>
</tr>
<tr>
<td>Range in days</td>
<td>—</td>
<td>7-9</td>
<td>6-8</td>
<td>—</td>
<td>5-10</td>
<td>11-13</td>
<td>40-48</td>
</tr>
</tbody>
</table>

Males and Females (30 individuals)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Egg</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days</td>
<td>6.00</td>
<td>8.10</td>
<td>6.67</td>
<td>6.00</td>
<td>6.90</td>
<td>12.40</td>
<td>46.07</td>
</tr>
<tr>
<td>Range in days</td>
<td>—</td>
<td>7-9</td>
<td>5-8</td>
<td>—</td>
<td>5-10</td>
<td>10-15</td>
<td>39-48</td>
</tr>
</tbody>
</table>

Fig. 4. Summary of life-history in out-of-door laboratory.

THE EFFECT OF FOOD UPON RATE OF GROWTH

Two types of food, namely, (1) growing milkweed plants and (2) dried seeds of milkweeds, were used in rearing cages placed side by side in the greenhouse.

Nymphs in the first instar, immediately after hatching, were placed in each cage. Those in the cage supplied with growing milkweed for food were not supplied with water. In the other cages, the diet of the nymphs consisted of dried milkweed pods and water. All other conditions except food were kept as nearly identical as possible. Table 7 shows a comparison of the rate of growth of the two groups.
TABLE 7. Comparison of development when fed on growing milkweed and ripe dried seeds of milkweed (water must be supplied with dried seeds)

<table>
<thead>
<tr>
<th>Food</th>
<th>Stage</th>
<th>Total days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>Green plants</td>
<td>5.71</td>
<td>6.00</td>
</tr>
<tr>
<td>Range in days</td>
<td>5-6</td>
<td>5-7</td>
</tr>
<tr>
<td>Dried Pods</td>
<td>5.26</td>
<td>6.00</td>
</tr>
<tr>
<td>Range in days</td>
<td>5-6</td>
<td>—</td>
</tr>
</tbody>
</table>

ADULT COPULATION

After the last molt the adults feed for several days before reaching sexual maturity, the length of the pre-mating period greatly depending upon the temperature and food conditions. When reared at a constant temperature of 34.5° C., the pre-copulation period varied from 6 to 8 days, at 29.5° C., from 7 to 13 days; and at 24.5° C., from 8 to 15 days. Dried milkweed pods were used as the sole source of food, and the relative humidity was kept at 70 per cent in each of the above cases.

The method of copulation is very similar to that of many other Hemiptera. The male usually approaches the female from one side, grasps her with his legs, and then attaches his genital organs. After the genital organs are firmly connected, the male loosens his hold with his feet and the bugs come to face in opposite directions. While in coitus the pair feed and move about on the plant, usually the female dragging the male. Normally copulation is repeated a number of times before egg-laying begins and may be repeated at numerous intervals during the entire period of egg deposition. The males are polygamic and the females polyandric. In the constant temperature cabinets the period of copulation lasted approximately 30 minutes at 38° C., 4 to 5 hours at 34.5° C., 12 to 30 hours at 30°., and for as long as 2 days at 24.5° C.

OVIPOSITION

Egg-laying begins from four to eight days after mating and may take place at any hour of the day or night. Under caged conditions the first
evidence of oviposition is noted by the female seeking a suitable place to
deposit eggs. After finding a wad of cotton and exploring it with the ros­
trum and ovipostor, the female comes to rest and lays from 2 to 38 eggs,
placing them well down in the cotton. Frequently the bug moves about
considerably while in the act of laying, thus tending to place eggs here
and there in the cotton.

When gravid females were confined in empty lamp chimneys, they
failed to oviposit until after a suitable medium such as a piece of cotton or
a fuzzy milkweed pod was added. In the field eggs may be deposited on
the underside of the leaves of milkweed plants in irregular masses. As
many as 30 eggs have been found on a single leaf.

The largest number of eggs are laid during the early part of the adult
stage (Fig. 6). In the case of 50 females which were reared at a constant
temperature of 29.5° C. and a relative humidity of 70 per cent, 25 females
laid the greatest number of eggs in the first batch. Of these 25 females
only 2 laid eggs on the day following, whereas in all but one case they
resumed laying on the second day following the first oviposition. Con­
siderable variation is shown by individual females in the total number of
eggs laid, the range being from as few as 5 to as many as 1238.

LONGEVITY OF ADULT

Very little is known regarding the duration of life, or “expectancy of
life,” of the members of the family Lygaeidae. Longevity in Oncopeltus
fasciatus is somewhat variable, the males usually living slightly longer
than the females. The total and mean length of adult life from the last
nymphal molt to death at a constant temperature of 34.5° C. ranged from
24 to 34 days for females and from 28 to 34 days for males, the mean being 27.93 days and 29.44 days, respectively; at 29.5° C., 22 to 35 days for females and 29 to 39 days for males, the mean being 32.75 days and 34.46 days, respectively; and at 24.5° C. the range in the case of females was from 28 to 35 days and 26 to 39 days in males, the mean being 30.72 days and 34.46 days, respectively.

**NATURAL ENEMIES**

**Predators.** Several species of predatory insects and a number of spiders frequently visit the milkweed plant in their quest for food. During the fruiting season the flowers and pods of milkweeds serve as suitable haunts in which predacious forms often secrete themselves. The ambush bug, *Phymata fasciata* (Gray), was encountered more frequently than any other insect preying on the larger milkweed lygaeid. On one occasion a female of a mating pair of phymatids was observed with its beak im¬paled in an adult. Two species of nabids, *Nabis ferus* (L.) and *Nabis rosepennis* Reuter, were taken a number of times while feeding on nymphs. A reduviid, *Sinea diadema* (Fab.), too, has not infrequently been found preying on both young and adults.

An orb-weaving spider very frequently spins its silken web on the milkweed plant, thus setting a treacherous trap for the larger milkweed lygaeid. Completely entangling the helpless bugs in the silken threads, the spider assures itself of a good supply of food. Nymphs as well as adults have been found on numerous occasions in the meshes of spiders’ webs.

**Cannibalistic Habits.** Although *Oncopeltus fasciatus*—both as nymphs and adults—is primarily a plant-feeding form, it sometimes betrays certain cannibalistic tendencies. Weakened or injured individuals, and especially nymphs during the process of molting, often fall victims to their own relatives. As many as seven nymphs in the same rearing cage have been observed feeding on one of their members during ecdysis.

**Parasites.** *Oncopeltus fasciatus* is recorded as the host of both an insect and a protozoan parasite. It is the insect host of *Herpetomonas elmassiani* (Migone), a protozoon which is found also in milkweed. This particular organism has been recorded by Holmes (1925 c) as inhabiting the three-lobed thoracic salivary gland. He observed that they were definitely localized in this gland, colonizing only the dorsal and anterior lobes.

Morrill (1910) has reported a Tachina parasite of adults. This fly normally lays its eggs about the head and thorax.

**HIBERNATION**

Although *Oncopeltus fasciatus* passes the winter in the adult stage in warm localities, such as California, the writer has been unable to find hibernating individuals in any stage in Iowa. Frequently adults of the genus *Lygaeus* and other related forms have been encountered, but the earliest record for *Oncopeltus* at Ames is July 7. Two cages were set up in a field over milkweed plants in the fall of 1932, and 50 adults and numerous nymphs were placed in each. The following spring all were dead. A rather interesting fact which developed from the examination of numerous specimens collected in various localities in Iowa over a period of years is that those specimens collected south of Ames along the Missouri border were often recorded as much as two weeks earlier during
the summer, while those collected north always appeared later than at Ames.

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VAN DUZEE, E. P.  


WEISS, H. B., AND E. L. DICKERSON  

WRIGHT, P. M.  

PLATE I

Fig. A. Adult female of Oncopeltus fasciatus (Dallas).

Fig. B. Eggs on milkweed leaf shortly after deposition.
PLATE I

A

B
VANADIUM OXYTRICHLORIDE AS A SOLVENT II
F. E. BROWN AND F. A. GRIFFITTS

From the Department of Chemistry, Iowa State College
Accepted for publication July 24, 1934

A previous paper\(^1\) reports data on the solubilities of about 125 substances in vanadium oxytrichloride. Recently Mellor\(^2\) has prepared a comprehensive review of the data and literature on vanadium oxytrichloride. The freezing point is given as "below \(-15^\circ\)". No vapor pressures are given except at the boiling point. The data on the interrelations of the metals and vanadium oxytrichloride is meager and some of the statements are contradictory or incomplete. The reactions with the alkali metals and white phosphorus are instances.

This paper deals especially with the relationships between the elements and vanadium oxytrichloride, but includes some other data.

PREPARATION OF VANADIUM OXYTRICHLORIDE

The vanadium oxytrichloride was prepared by the method used by Briscoe and Little\(^9\), when they were determining the atomic weight of vanadium, with one change in procedure. Much trouble was experienced in chlorinating the lower oxide obtained by passing hydrogen over hot vanadium pentoxide because of the caking of the oxide. The powdery reduced material became glassy and adhered to the walls of the glass tubes at temperatures below that required for chlorination. When the method of Briscoe and Little was modified by mixing the lower oxide with powdered charcoal, before its introduction into the tube for chlorination, no difficulty was experienced. It was then possible to use the high temperatures produced by Meeker burners and increase the rate of formation of the compound. The charcoal was never entirely consumed and may have been useful only in maintaining porosity. About 1,450 cc. of the crude product was prepared. After refluxing it over sodium and fractionally distilling it, about 900 cc. of a light yellow liquid whose analysis showed it to be 99.72 per cent VOCl\(_3\) was secured. This was used in the experimental work reported in this paper.

EXPERIMENTAL PHYSICAL PROPERTIES

The boiling point was 124.5\(^\circ\) at 736 mm. pressure. The density was 1.854 at 0\(^\circ\) and 1.811 at 32\(^\circ\). All attempts to freeze it failed. The lowest temperature tried was \(-77^\circ\). At low temperatures two phases appeared. One, the smaller in amount, was reddish in color and appeared to be a stringy clot of solid or extremely viscous substance.

The vapor pressure was determined over the range 
-77° to +80° and the boiling points at 736 mm. and at 
760 mm. are known. Figure 1 is a drawing of the apparatus used. The open manometer, about 120 mm. in length, 
was filled to about one-half of its length with mercury. The bulb B was evacuated and permitted to refill with air 
slowly through a drying train five times. Approximately 
25 cc. of freshly redistilled vanadium oxytrichloride was 
introduced into B through a tube which was drawn fine 
enough to pass through the stop-cock and reach to the 
bottom of the bulb. The vacuum pump was again attached 
to the tube above the bulb and evacuation continued un­
til about one-half of the liquid had been evaporated at 
room temperature. During the evacuation the upper end 
of the manometer was cooled until liquid vanadium oxy­
trichloride formed above the mercury. Subsequent warm­
ing permitted the liquid to evaporate and its vapor to 
sweep the air from the manometer. After the air had 
been displaced in this manner, the tube was sealed off 
below the stop-cock. Readings of mercury level in the 
manometer were made with a cathetometer. The greatest 
error in determining the vapor pressure was due to the 
fact that vanadium oxytrichloride causes mercury to wet 
glass. It was found impossible to make satisfactory read­
ings with decreasing pressure. Table 1 is a record of the 
data taken on vapor pressures.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Vapor pressure (mm.)</th>
<th>Temperature</th>
<th>Vapor pressure (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-77</td>
<td>4.1</td>
<td>40</td>
<td>60.9</td>
</tr>
<tr>
<td>-40</td>
<td>12.5</td>
<td>45</td>
<td>73.3</td>
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<tr>
<td>-15</td>
<td>17.5</td>
<td>50</td>
<td>89.5</td>
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<tr>
<td>-5</td>
<td>20.6</td>
<td>55</td>
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</tr>
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<td>75</td>
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<td>20</td>
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<td>80</td>
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<td>41.9</td>
<td>85</td>
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<td>30</td>
<td>47.8</td>
<td>124.5</td>
<td>736.0</td>
</tr>
<tr>
<td>35</td>
<td>56.5</td>
<td>127.16</td>
<td>780</td>
</tr>
</tbody>
</table>

Figure 2 is a graph formed by plotting vapor pres­
sure of vanadium oxytrichloride against temperature.

SOLUBILITIES OF ELEMENTS

The solubilities in and reactions of elements with 
v anadium oxytrichloride were determined by putting a 
small, carefully dried piece of each element which was available into 
about 5 cc. of the liquid. Observations were made for 10 to 15 days. Then
the temperature was raised to 125°. The following elements did not dissolve noticeably and did not show any indication of reaction under these conditions:

- Aluminum
- Barium
- Beryllium
- Bismuth
- Boron
- Cadmium
- Carbon
- Cerium
- Chromium
- Cobalt
- Columbium
- Copper
- Gold
- Hydrogen
- Iridium
- Iron
- Lead
- Magnesium
- Manganese
- Misch Metal
- Nickel
- Nitrogen
- Osmium
- Oxygen
- Phosphorus (red)
- Platinum
- Rhodium
- Ruthenium
- Silicon
- Silver
- Tellurium
- Thallium
- Tin
- Titanium
- Tungsten
- Uranium
- Zinc
- Zirconium

The following elements dissolve with little or no reaction at room temperatures: Bromine, chlorine, iodine, mercury, selenium, sulfur and vanadium. The last four of this list may react slightly at room temperatures. Sulfur certainly does react at higher temperatures.

The quantitative solubility of iodine was determined at six points between 0° and 100°. A flask containing a supply of vanadium oxytrichloride was kept in contact with an excess of iodine for 12 to 15 hours at the chosen temperature. Then 5 cc. was pipetted into 250 cc. of water. After adding an excess of ferric alum and acidifying with sulfuric acid, the iodine was distilled into a solution of potassium iodide and titrated with thiosulfate. The solubility of iodine in vanadium oxytrichloride expressed as grams of iodine in 5 cc. of solution is reported in table 2.

**TABLE 2. Solubility of iodine in vanadium oxytrichloride**

<table>
<thead>
<tr>
<th>Temperature degrees C.</th>
<th>I</th>
<th>II</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.0957</td>
<td>0.0820</td>
<td>.08835</td>
</tr>
<tr>
<td>25</td>
<td>0.4511</td>
<td>0.4237</td>
<td>.04374</td>
</tr>
<tr>
<td>47</td>
<td>1.2439</td>
<td>1.3123</td>
<td>1.27610</td>
</tr>
<tr>
<td>67</td>
<td>2.1872</td>
<td>2.2145</td>
<td>2.20085</td>
</tr>
<tr>
<td>92</td>
<td>3.1099</td>
<td>3.0416</td>
<td>3.07575</td>
</tr>
<tr>
<td>100</td>
<td>4.1935</td>
<td>4.2960</td>
<td>4.24475</td>
</tr>
</tbody>
</table>

Fig. 2. Vapor pressure of vanadium.
Solubility of Arsenic

Arsenic reacts slightly with vanadium oxytrichloride. An excess of arsenic was added to a sample of vanadium oxytrichloride and let stand for some time, after which the arsenic in the supernatant liquid at 25° C. was determined.

0.6731 gms. contained 0.1485 gms. of arsenic
0.6569 gms. contained 0.1674 gms. of arsenic

These figures represent a solubility of 0.2206 gram and 0.2507 gram of arsenic per gram of vanadium oxytrichloride, respectively.

The following elements react with vanadium oxytrichloride at room temperature and in some cases with explosive violence: antimony, arsenic, caesium, calcium, gallium, indium, phosphorus (white), potassium, rubidium and sodium.

Nearly all investigators who have worked with this liquid have purified it by refluxing it over sodium. Brown and Snyder\(^4\) reported that it may be refluxed over sodium for several days without suffering appreciable reduction or decomposition, that the metals potassium and sodium are unaffected at room temperature or at the boiling point of vanadium oxytrichloride, and that white phosphorus dissolves at room temperature without apparent reaction. In all three of these cases the report is misleading or entirely in error.

The statements regarding potassium and phosphorus were not accurately transcribed in condensing the original longer paper. Page 7 of Snyder's original thesis, on file in Iowa State College Library, contains the sentences: "Potassium reacts violently at about 90°." "White phosphorus dissolves without apparent reaction at room temperature but reacts vigorously at temperatures a little below the boiling point of water."

But these statements are not entirely correct. A bright piece of potassium darkens and collects a black deposit of what appears to be lower oxides of vanadium, from vanadium oxytrichloride, even at ordinary room temperature, and reacts explosively at about 100°.

Small pieces of white phosphorus do disappear in vanadium oxytrichloride without producing any residue or disturbance at room temperature. But large proportions of phosphorus produce rapid rises in temperature and violent and even explosive reactions, even when the contact between the two is effected at room temperature. Arsenic and antimony react with much less vigor than phosphorus, and bismuth does not react. The vigor of the reaction decreases with increasing atomic weight for these four elements.

An explosion occurred just before the distillation of a sample of vanadium oxytrichloride from sodium was completed. This led to closer observation of the interaction between this liquid and sodium. When the two were left in contact at room temperatures the bright metal became dull and later a noticeable black deposit appeared. Pieces of sodium and about 10 cc. of the liquid were sealed in a combustion tube and placed in a bomb furnace. The temperature was gradually raised. At 180°-185° the tube was shattered by an explosion. Lower oxides of vanadium and sodium chloride were recovered from the furnace. A second tube simi-

larly filled was heated to 175° and cooled. Much of both sodium and vanadium oxytrichloride in it remained unchanged, but the sodium was covered with a layer of black substance.

The approximate temperatures of violent reaction between liquid vanadium oxytrichloride and the alkali metals are: caesium 30°, rubidium 60°, potassium 100°, sodium 180°, lithium not determined. Calcium and indium react very slightly. Gallium reacts to form a blue precipitate.

**REACTIONS WITH SULFUR DIOXIDE HYDROGEN SULFIDE AND GRIGNARD REAGENT**

Liquid sulfur dioxide is immiscible and no reactions occur between these two substances in either the liquid or the gaseous state.

When liquid hydrogen sulfide and vanadium oxytrichloride are mixed a precipitate forms. One precipitate contained: vanadium 27.78 per cent, sulfur 20.14 per cent, and chlorine 33.08 per cent. The compositions of other precipitates varied, but were between that of the formulas for VSCl$_3$ and VOCl. H$_2$S, or VOHSHCl.$^4$

When phenyl magnesium bromide and vanadium oxytrichloride were mixed no stable vanadium compound was formed. Vernon$^6$ reports the formation of a green organic compound by this method. The compound darkens at 85-100°, does not melt up to 220°, cannot be crystallized from any common organic solvent, burns in flashes of sooty yellow flame and leaves a residue containing vanadium.

**SUMMARY**

1. About 900 cc. of pure vanadium oxytrichloride were prepared by the action of chlorine on vanadium trioxide in the presence of finely divided charcoal. This method gave an excellent yield.

2. The freezing point was found to be at least 60° C. below that previously reported.

3. The vapor pressure from $-77°$ to $+80°$ was determined.

4. About 50 elements were found to be insoluble and non-reactive.

5. The quantitative solubilities of iodine and of arsenic were determined.

6. The reactions of sodium, potassium, rubidium, caesium, phosphorus, arsenic and antimony were studied.

7. The compound reacts with liquid hydrogen sulfide, but does not react with liquid sulfur dioxide.

8. It does not form stable vanadium organic compounds with the phenyl magnesium bromide.

---


The effect of the application of nitrogenous fertilizers to apple trees on the chemical make-up of the fruit and its metabolic activity, and the relationship of these to keeping quality in storage, is a subject of acute controversy in the fruit growing and storage industries. The use of nitrogen carrying fertilizers is frequently questioned when apples which have received such treatment break down prematurely, and it is assumed that the treatment predisposes the fruit to breakdown or other related diseases. Such an assumption has some justification on the basis that the results of certain research work have led to the conclusion that the nitrogen content of the apple may regulate metabolic activity in storage and that, therefore, the proportion of nitrogen to the storage reserves in the form of carbohydrates and organic acids determines the storage life of the fruit. Much of the earlier work on apple storage was done before the use of fertilizing materials was adopted as a standard practice, and, since more fruit is being produced under a system which includes the application of nitrogenous fertilizers, the question of their effect on stored fruit is becoming of increasing importance.

In the work herein reported the writer has undertaken to determine whether there is a casual relationship between the nitrogen content of the apple and susceptibility to soggy breakdown; whether the relationship between the sugar and nitrogen content of the fruit is affected by sodium nitrate applications to the trees; and whether this relationship manifests itself in the susceptibility to soggy breakdown.

Archbold (2) and Kidd and West (11) found a correlation to exist between nitrogen content and respiratory activity in apples, and storage tests showed low nitrogen fruit to keep longer than high nitrogen fruit. Gourley and Hopkins (6) and Aldrich (1) found that nitrogenous fertilizers increased the nitrogen content of the apple, but not its susceptibility to breakdown in storage. Degman (4), Weinberger (19) and Knowlton and Hoffman (12) found no correlation to exist between nitrogen fertilization and storage quality in apples. The results of Overley and Overholser (14), on the other hand, indicated that nitrogen fertilizers increased the susceptibility to Jonathan breakdown, particularly in seasons when the trouble was common. Harding (7) found a correlation between the nitrogen fertilization of Grimes apples and their respiratory activity.

SOURCE AND TREATMENT OF MATERIAL

The experimental fruit for the 1929-30 storage season, in the case of nitrated trees, was obtained from 25-year-old trees which had received...
nitrate of soda applications in 1927, 1928 and 1929; in the case of the check trees, from trees of a nearby orchard which had never received fertilizer. For convenience, the orchard with the different nitrate treatments will be hereafter referred to as orchard A, and the one with only check treatments as orchard B. The fertilized orchard was located in a loess type of soil which is considered well adapted for fruit trees, while the check orchard lay in a gravelly type of soil of less uniform texture and was less retentive of moisture. Treatments in the fertilized orchard included trees which were not fertilized after 1928, so that in a sense it also contained check treatments. For the storage seasons of 1930-31 and 1931-32, the fruit was obtained from the same trees as before. These trees received the same treatment continually and comparable storage fruit was obtained from each treatment each year.

These treatments in the fertilized orchard varied in the nitrate applied each year. In treatment I, the four trees each received five pounds of nitrate in 1928; in treatment II, each received five pounds in 1927 and 1928; in treatment III, each received five pounds in 1927, 1928 and 1929; in treatment IV, each received five pounds in 1927 and 1928, and ten pounds in 1929. In 1930 and 1931, additional five and ten pound applications were given to treatments III and IV, respectively. Treatment I received no application in 1930 and 1931, while treatment II received a five pound application in 1931. The following tabulation shows the arrangement and status of each treatment for the three years of the experiment:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Orchard</th>
<th>1929</th>
<th>1930</th>
<th>1931</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fertilized</td>
<td>0-5-5</td>
<td>0-5-0-0</td>
<td>0-5-0-0-0</td>
</tr>
<tr>
<td>II</td>
<td>&quot;</td>
<td>5-5-0</td>
<td>5-5-0-0</td>
<td>5-5-0-0-5</td>
</tr>
<tr>
<td>III</td>
<td>&quot;</td>
<td>5-5-5</td>
<td>5-5-5-5</td>
<td>5-5-5-5-5</td>
</tr>
<tr>
<td>IV</td>
<td>&quot;</td>
<td>5-5-10</td>
<td>5-5-10-10</td>
<td>5-5-10-10-10</td>
</tr>
<tr>
<td>V</td>
<td>Check</td>
<td>0-0-0</td>
<td>0-0-0-0</td>
<td>0-0-0-0-0</td>
</tr>
</tbody>
</table>

In the above table, the numerals 0, 5 and 10 indicate the nitrate fertilizer in pounds per tree applied in different years and do not refer to other mineral nutrients. The only fertilizer used in this investigation was nitrate of soda. For convenience, treatments I and II will be referred to as the minus nitrogen treatments; treatment III as the normal nitrogen treatment; treatment IV as the plus nitrogen treatment.

The storage treatment was comparable during each of the three seasons. Grimes and Jonathan apples were stored in standard boxes in duplicate lots at temperatures of 30-31° F., 35-36° F. and 48-50° F. Lots given immediate treatment were stored the day of picking, while deferred lots were temporarily stored in the 48-50° F. room until the time designated for storage at lower temperature. The methods used for temperature and humidity control have been described in a previous publication (16). Preliminary inspections were made within the storage rooms and the final condition of the fruit was determined early in March each season.
METHODS OF SAMPLING AND ANALYSES

Samples of fruit were analyzed for non-colloidal, colloidal and total nitrogen; for reducing, non-reducing and total sugars; and for alcohol insoluble residue. The first year the sampling began on August 1, while the fruit was green, and continued bi-weekly until the commercial picking date, and thereafter bi-weekly from various storage lots. During the second and third years the chemical investigations were confined to samples of fruit taken on the date of picking and after the normal storage period. The variety Grimes Golden was used for most of the work, but many analyses were also made on Jonathan. The samples consisted of duplicate 100-gram lots of thinly sliced tissue from the centers of 20 apples of medium size selected from the tree and storage lots. These were preserved in alcohol. Extraction was by the decantation method. Non-colloidal and colloidal nitrogen was determined from the extract and alcohol insoluble residue, respectively by the official Kjeldahl method. Reducing and non-reducing sugars were determined from the cleared extract by the Munson-Walker-Bertrand volumetric method, and alcohol insoluble residue by weighing the dried residue remaining after extracting.

RESULTS OF STORAGE TESTS

The storage results for the three seasons for both Grimes and Jonathan are given in table 1. The data for the first season indicate that the nitrogen treatments increased the susceptibility of the fruit to soggy breakdown. However, in the following seasons (1930 and 1931), which were unusually dry, not enough breakdown occurred in any of the storage treatments to give conclusive results. The data suggest that dry weather was the controlling factor in 1930 and 1931, rather than nitrate fertilization. The investigations on Jonathan breakdown of Overley and Overholser (14) and of Palmer (15) indicate clearly seasonal differences in susceptibility, and the former investigators (l.c.) pointed out that nitrogen fertilization increased susceptibility in seasons when the disease was common. Recent studies by Plagge and Maney (16) also lead to similar conclusions.

RESULTS OF ANALYSES

The results of the analyses of the fruit will be considered under separate headings, i.e., nitrogen, sugar and insoluble residue. In each case, the data have been summarized and are representative of the general results obtained. For detailed reports of individual analyses, the reader is referred to the original thesis by the author (17).

NITROGEN

Table 2 gives the nitrogen content of Grimes and Jonathan apples on the picking dates of the three seasons. The data show the results for the nitrated and check treatments of fertilized and check orchards, respectively. The differences between the two treatments in 1929 were slight and fruit from the check trees had approximately the same nitrogen content (including both non-colloidal and colloidal forms), as that from nitrated trees. However, in 1930 and 1931 fruit from fertilized trees of both Grimes and Jonathan was consistently higher in total nitrogen. The differences were principally in non-colloidal nitrogen.
TABLE 1. Effect of nitrate fertilizer and storage treatment on soggy breakdown in Grimes and Jonathan. Percentage of breakdown at 30-31° F.

<table>
<thead>
<tr>
<th>Treatment before storing</th>
<th>Grimes</th>
<th></th>
<th>Jonathan</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Season 1929-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stored directly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0</td>
<td>2.5</td>
<td>9.8</td>
<td>11.1</td>
<td>2.5</td>
</tr>
<tr>
<td>0-5-0</td>
<td>4.6</td>
<td>47.7</td>
<td>29.2</td>
<td>4.6</td>
</tr>
<tr>
<td>5-5-0</td>
<td>14.6</td>
<td>77.5</td>
<td>53.8</td>
<td>14.6</td>
</tr>
<tr>
<td>5-5-5</td>
<td>4.7</td>
<td>76.2</td>
<td>41.0</td>
<td>4.7</td>
</tr>
<tr>
<td>5-5-10</td>
<td>5.0</td>
<td>66.2</td>
<td>43.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Deferred 10 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deferred 20 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5-0</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5-5-0</td>
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<tr>
<td>5-5-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season 1930-31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stored directly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0-0*</td>
<td>1.4</td>
<td>0.0</td>
<td>7.5</td>
<td>1.4</td>
</tr>
<tr>
<td>0-5-0-0</td>
<td>3.9</td>
<td>0.3</td>
<td>0.0</td>
<td>3.9</td>
</tr>
<tr>
<td>5-5-0-0</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
<td>2.2</td>
</tr>
<tr>
<td>5-5-5-5</td>
<td>2.4</td>
<td>0.0</td>
<td>0.0</td>
<td>2.4</td>
</tr>
<tr>
<td>5-5-10-10</td>
<td>8.8</td>
<td>0.0</td>
<td>0.0</td>
<td>8.8</td>
</tr>
<tr>
<td>Deferred 10 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0-0*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5-0-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-0-0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>5-5-5-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-10-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deferred 20 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0-0-0*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-5-0-0</td>
<td></td>
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</tr>
<tr>
<td>5-5-0-0</td>
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<tr>
<td>5-5-5-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-5-10-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numerals 0, 5 and 10 indicate the amount of nitrate fertilizer in pounds per tree applied during different years. Thus: 0-5-0 indicates no application in 1927, 5 pounds in 1928 and no application in 1929; while 5-5-10-10 indicates an application of 5 pounds per tree each year for 1927 and 1928, and 10 pounds per tree each for the years 1929 and 1930.
### TABLE 2. Nitrogen content of apples in different seasons in samples analyzed on the commercial picking date.

#### Grimes

<table>
<thead>
<tr>
<th>Year</th>
<th>Non-colloidal N. (mg.)</th>
<th>Colloidal N. (mg.)</th>
<th>Total N. (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>1929</td>
<td>16.14</td>
<td>19.52</td>
<td>24.32</td>
</tr>
<tr>
<td>1930</td>
<td>14.53</td>
<td>23.00</td>
<td>19.93</td>
</tr>
<tr>
<td>1931</td>
<td>8.79</td>
<td>22.54</td>
<td>22.62</td>
</tr>
</tbody>
</table>

#### Jonathan

<table>
<thead>
<tr>
<th>Year</th>
<th>Non-colloidal N. (mg.)</th>
<th>Colloidal N. (mg.)</th>
<th>Total N. (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>1929</td>
<td>18.30</td>
<td>15.97</td>
<td>22.45</td>
</tr>
<tr>
<td>1930</td>
<td>11.05</td>
<td>18.05</td>
<td>20.01</td>
</tr>
<tr>
<td>1931</td>
<td>8.64</td>
<td>19.76</td>
<td>14.92</td>
</tr>
</tbody>
</table>

### TABLE 3. Effect of varying the application of nitrate of soda on the nitrogen content of the fruit. Season 1929-30. Storage temperature 30°-31° F. Fruit stored immediately.

#### Grimes

<table>
<thead>
<tr>
<th>Date of</th>
<th>Non-colloidal nitrogen (mg.)</th>
<th>Plus nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 26*</td>
<td>0.0-0.0</td>
<td>0.5-0.0</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>0.0-0.0</td>
<td>0.5-0.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of</th>
<th>Colloidal nitrogen (mg.)</th>
<th>Plus nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan. 6</td>
<td>27.733</td>
<td>21.446</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of</th>
<th>Total nitrogen (mg.)</th>
<th>Plus nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 26*</td>
<td>40.460</td>
<td>31.920</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>40.810</td>
<td>26.110</td>
</tr>
</tbody>
</table>

*Picking date.
The data in table 3 show the effect of the various nitrate treatments within the fertilized orchard on the nitrogen content of Grimes apples during the seasons 1929-30. Lower nitrogen values for the low nitrate treatments (0-5-0 and 5-5-0) were consistent for both the non-colloidal and colloidal forms, at the end as well as at the beginning of the storage period. Non-colloidal nitrogen decreased considerably in fruit from the low nitrogen treatments of the fertilized orchard, while colloidal nitrogen remained the same. There was no marked decrease in non-colloidal nitrogen during storage in either of the samples from the check treatment of the unfertilized orchard or from the high nitrogen treatment. The similarity in nitrogen content between samples from these two treatments noted on the picking date was again apparent after storage.

The nitrogen content of Grimes fruit in 1930, as affected by various nitrate treatments, is shown in table 4. In this year nitrate fertilization consistently increased the total nitrogen content of the fruit and, in most instances, both non-colloidal and colloidal nitrogen content. The most marked increases of nitrated over unnitrated samples were in the non-colloidal form. The low nitrate treatments of the fertilized orchard were approximately of the same total nitrogen content as the treatments of the unfertilized orchard, while the normal (5-5-5-5) and plus (5-5-10-10) TABLE 4. Effect of varying the application of nitrate of soda on the nitrogen content of the fruit. Season 1930-31. Fruit stored immediately in cold storage

Grimes

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Stor. temp. F.</th>
<th>Non-colloidal nitrogen (mg.)</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td>11.945</td>
<td>5.185</td>
<td>4.509</td>
<td>9.694</td>
<td>26.500</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Stor. temp. F.</th>
<th>Colloidal nitrogen (mg.)</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td>29.021</td>
<td>22.257</td>
<td>21.063</td>
<td>26.489</td>
<td>31.190</td>
<td></td>
</tr>
<tr>
<td>April 1</td>
<td>35°-36°</td>
<td>24.051</td>
<td>20.054</td>
<td>23.109</td>
<td>28.749</td>
<td>32.126</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Stor. temp. F.</th>
<th>Total nitrogen (mg.)</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 24</td>
<td>*</td>
<td>34.461</td>
<td>30.722</td>
<td>30.626</td>
<td>42.117</td>
<td>49.720</td>
<td></td>
</tr>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td>40.966</td>
<td>27.442</td>
<td>25.752</td>
<td>36.183</td>
<td>57.690</td>
<td></td>
</tr>
<tr>
<td>April 1</td>
<td>35°-36°</td>
<td>36.756</td>
<td>23.962</td>
<td>27.843</td>
<td>38.292</td>
<td>48.026</td>
<td></td>
</tr>
</tbody>
</table>

*Picking date.
nitrate treatments yielded fruit considerably higher in nitrogen content. Non-colloidal nitrogen decreased during storage under the low and normal nitrate treatments at both storage temperatures 30-31° F. and 35-36° F.

A study of the nitrogen content of apples during the storage period was made. The nitrogen content of Grimes was determined approximately at monthly intervals in 1929-30. The results obtained for storage at 48-50° F. are given in tables 5 and 6, respectively. These data show that non-colloidal nitrogen decreased rapidly in untreated fruit during the first 20 days of storage at 48-50° F. Fruit from the check treatments decreased approximately 60 per cent, while that from the nitrate treatment decreased only 18 per cent in non-colloidal nitrogen. In table 6 it will be noted that non-colloidal nitrogen was higher in fruit from the fertilized trees throughout the storage period, because of the more rapid loss in the apples from unfertilized trees during the first three weeks of storage at 48-50° F. This point is of interest in connection with the nitrogen analyses of peaches reported by Nightingale et al (13), who found that unfertilized fruits had a higher percentage of nitrogen present in the protein-like form, whereas fruit from nitrated trees which had more nitrogen present had most of it in the simpler amino acid form. On the other hand, Thomas (18)

### TABLE 5. Nitrogen content of Grimes. Season 1929-30. Fruit stored immediately at 48°-50° F.

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Non-colloidal N. mg.</th>
<th>Colloidal N. mg.</th>
<th>Total N. mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>7.060</td>
<td>11.670</td>
<td>20.263</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>3.890</td>
<td>13.975</td>
<td>25.590</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>6.689</td>
<td>11.650</td>
<td>24.857</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Non-colloidal N. mg.</th>
<th>Colloidal N. mg.</th>
<th>Total N. mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>8.644</td>
<td>9.941</td>
<td>21.983</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>8.493</td>
<td>15.032</td>
<td>23.776</td>
</tr>
<tr>
<td>March 15</td>
<td>8.268</td>
<td>18.940</td>
<td>25.026</td>
</tr>
</tbody>
</table>

*Picking date.
**Storage date.
found a close parallelism between total water soluble nitrogen and free amino nitrogen throughout the cycle in all tissues of the apple, and suggested the possibility that amino acids are the chief fractions influencing growth and vigor of the tree.

Therefore, it seems that the non-colloidal nitrogen fraction, as determined in this investigation, may be the important factor in regulating the metabolism of the apple, and thus offer an explanation for greater susceptibility to soggy breakdown in apples from fertilized trees. The non-colloidal nitrogen content of fertilized fruits was over 100 per cent higher in Grimes after three weeks of deferred storage and continued thus after five and one-half months in storage at 30-31° F.

This investigation as carried out offered an opportunity to observe whether there is a residual effect in the nitrogen content of the tree resulting from nitrate fertilization, and whether this is reflected in the nitrogen content of the fruit. In table 3, the data for Grimes in 1929 show that there was a greater proportion of non-colloidal and total nitrogen under treatment 5-5-0 as compared with treatment 0-5-0, which indicates that the 1927 application of nitrate increased the nitrogen content of the fruit in the 1929 year. However, the results for 1930 (table 4) do not indicate that a residual nitrogen content, if present in the tree, influenced the nitrogen content of the fruit. Treatments 0-5-0-0 and 5-5-0-0 were about the same in nitrogen content; in other words, the 1927 nitrate application did not appear to affect the nitrogen content of the fruit in 1930. It appears, therefore, that nitrate fertilizers increased the nitrogen content of the fruit the first and second years after applying, but not in the third year. The principal differences were in the non-colloidal form.

SUGAR

The sugar content of Grimes apples before commercial harvest was studied in 1929. The percentage of sugar for both the check and high nitrogen treatments for samples picked at intervals during ripening is given in table 7. It will be noted that reducing and total sugars were consistently lower in the high nitrogen samples, with the exception of the first sample taken on August 1. The same was true for non-reducing sugars except for one analysis.

TABLE 7. Percentage of sugar in Grimes. Season 1929-30. Early picked fruit

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Reducing sugars</th>
<th>Non-reducing sugars</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Aug. 1</td>
<td>4.74</td>
<td>5.11</td>
<td>1.61</td>
</tr>
<tr>
<td>Aug. 15</td>
<td>5.46</td>
<td>4.84</td>
<td>3.13</td>
</tr>
<tr>
<td>Sept. 5</td>
<td>5.86</td>
<td>5.01</td>
<td>2.42</td>
</tr>
<tr>
<td>Sept. 16</td>
<td>5.63</td>
<td>5.08</td>
<td>3.73</td>
</tr>
<tr>
<td>Sept. 26*</td>
<td>5.79</td>
<td>5.77</td>
<td>4.81</td>
</tr>
</tbody>
</table>

*Commercial picking.

The results of analyses taken on fruit stored immediately at 30-31° F. in 1929 are shown in table 8. In this case total and non-reducing sugars
are usually higher in the fruit from the check trees. The results give a picture of the sugar content throughout the storage period at 30-31° F.

**TABLE 8. Percentage of sugar in Grimes. Season 1929-30. Fruit stored immediately at 30°-31° F.**

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Reducing sugars</th>
<th>Non-reducing sugars</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>5.79</td>
<td>5.77</td>
<td>4.81</td>
</tr>
<tr>
<td>Oct. 17</td>
<td>6.06</td>
<td>6.05</td>
<td>4.89</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>6.15</td>
<td>6.16</td>
<td>5.10</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>6.63</td>
<td>6.59</td>
<td>4.88</td>
</tr>
<tr>
<td>March 15</td>
<td>7.24</td>
<td>8.26</td>
<td>4.06</td>
</tr>
</tbody>
</table>

The sugar content of Grimes apples throughout the storage period at 48-50° F. is shown in table 9. In this case, again a slightly higher total sugar level in the fruit from the unfertilized trees was maintained throughout the storage period. Sucrose content also was consistently higher in the check fruit, while reducing sugar showed an increase in the nitrated fruit during the latter part of the season.

**TABLE 9. Percentage of sugar in Grimes. Season 1929-30. Fruit stored immediately at 48°-50° F.**

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Reducing sugars</th>
<th>Non-reducing sugars</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>5.79</td>
<td>5.77</td>
<td>4.81</td>
</tr>
<tr>
<td>Oct. 17</td>
<td>6.14</td>
<td>5.95</td>
<td>5.76</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>6.64</td>
<td>6.73</td>
<td>5.43</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>6.94</td>
<td>7.06</td>
<td>5.21</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>7.38</td>
<td>8.29</td>
<td>4.32</td>
</tr>
</tbody>
</table>

The data in table 10 show that there was a higher sugar level in check fruit during the second year of the investigation. These data indicate that total sugars and sucrose were slightly higher in check fruit, as well as in the minus nitrogen fruit from the fertilized orchard. Reducing sugars fluctuated more than non-reducing sugar; the latter decreased, while the former increased during storage.

It has been pointed out that 1930 and 1931 were below normal in rainfall. The year 1929 was almost average in the amount of rainfall, while 1928 was excessively wet. Analyses of both Grimes and Jonathan apples on the picking date were made in 1931, so that a comparison of the sugar content for the three years 1929, 1930 and 1931 is possible. The data in table 11 show these results. Total sugars in orchard A Grimes in the two dry years (1930 and 1931) were slightly higher, but in the case of orchard B fruit, the results were less consistent. Likewise, with Jonathan, total sugars were not consistently higher in 1930 and 1931. Non-reducing
TABLE 10. Effect of various nitrate applications on percentage of sugar in Grimes. Season 1930-31. Fruit stored immediately

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Storage temp. F.</th>
<th>Percentage of reducing sugars plus nitrogen</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 24</td>
<td></td>
<td></td>
<td></td>
<td>6.29</td>
<td>7.11</td>
<td>7.05</td>
<td>7.36</td>
</tr>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td></td>
<td></td>
<td>8.48</td>
<td>8.32</td>
<td>8.28</td>
<td>8.42</td>
</tr>
<tr>
<td>April 1</td>
<td>35°-36°</td>
<td></td>
<td></td>
<td>8.61</td>
<td>8.64</td>
<td>7.84</td>
<td>8.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Storage temp. F.</th>
<th>Percentage of non-reducing sugars plus nitrogen</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 24</td>
<td></td>
<td></td>
<td></td>
<td>4.08</td>
<td>3.84</td>
<td>3.91</td>
<td>2.94</td>
</tr>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td></td>
<td></td>
<td>3.03</td>
<td>3.47</td>
<td>2.75</td>
<td>3.39</td>
</tr>
<tr>
<td>April 1</td>
<td>35°-36°</td>
<td></td>
<td></td>
<td>3.24</td>
<td>2.84</td>
<td>3.01</td>
<td>2.86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>Storage temp. F.</th>
<th>Percentage of total sugars plus nitrogen</th>
<th>Check</th>
<th>0-5-0-0</th>
<th>5-5-0-0</th>
<th>5-5-5-5</th>
<th>5-5-10-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 24</td>
<td></td>
<td></td>
<td></td>
<td>10.37</td>
<td>10.95</td>
<td>10.96</td>
<td>10.30</td>
</tr>
<tr>
<td>April 1</td>
<td>30°-31°</td>
<td></td>
<td></td>
<td>11.52</td>
<td>11.79</td>
<td>11.02</td>
<td>11.81</td>
</tr>
<tr>
<td>April 1</td>
<td>35°-36°</td>
<td></td>
<td></td>
<td>11.85</td>
<td>11.48</td>
<td>10.86</td>
<td>10.91</td>
</tr>
</tbody>
</table>

*Picking date.

sugars were correspondingly lower and reducing sugars higher in Grimes fruit in the two dry years, but a similar relationship did not hold for Jonathan. We may conclude, therefore, that dry weather in 1930 and 1931 did not increase the sugar content of apples over that of 1929.

The data on sugar analyses in the above tables are considered to be evidence that nitrate fertilization of apple trees may result in slightly lowering the total sugar and non-reducing sugar content of the apple. Still other data obtained with Grimes apples under other storage treatment and further data on Jonathan apples might be cited, but these would only emphasize what has already been indicated in the above tables. The results are in harmony with those reported by Hopkins and Gourley (9) and Hopkins and Greve (10), who in a similar investigation found small but consistent differences in the soluble carbohydrates between apples from nitrated and unnitrated trees. The results herein reported show that the most marked changes during storage were in sucrose content. In a few instances sucrose reached a very low level in fruit from the nitrated trees. This was especially true in the case of fruit stored at the higher temperature (48-50° F.). During the storage period reducing-
TABLE 11. Percentage of sugar in apples in different seasons. Analyses on the commercial picking date

Grimes

<table>
<thead>
<tr>
<th>Year</th>
<th>Reducing sugars</th>
<th>Non-reducing sugars</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>1929</td>
<td>5.79</td>
<td>5.77</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td>4.03</td>
<td>10.61</td>
<td>9.80</td>
</tr>
<tr>
<td>1930</td>
<td>6.29</td>
<td>7.01</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>3.29</td>
<td>10.37</td>
<td>10.30</td>
</tr>
<tr>
<td>1931</td>
<td>6.42</td>
<td>6.37</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>10.92</td>
<td>10.12</td>
</tr>
</tbody>
</table>

Jonathan

<table>
<thead>
<tr>
<th>Year</th>
<th>Reducing sugars</th>
<th>Non-reducing sugars</th>
<th>Total sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>1929</td>
<td>7.57</td>
<td>6.12</td>
<td>3.51</td>
</tr>
<tr>
<td></td>
<td>4.14</td>
<td>11.08</td>
<td>10.27</td>
</tr>
<tr>
<td>1930</td>
<td>8.16</td>
<td>7.62</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td>2.38</td>
<td>11.21</td>
<td>9.99</td>
</tr>
<tr>
<td>1931</td>
<td>7.24</td>
<td>7.67</td>
<td>3.70</td>
</tr>
<tr>
<td></td>
<td>4.04</td>
<td>10.94</td>
<td>11.71</td>
</tr>
</tbody>
</table>

sugar values indicated more stability than sucrose values, and this result is in accordance with those of Evans (5), who found reducing sugar content in apples to fluctuate within a narrower margin than sucrose and total sugar contents. These results suggest that reducing sugars are supplied to the apple (by hydrolysis of sucrose) about as fast as they are being oxidized.

THE RATIO OF SUGAR TO NITROGEN AS AN INDICATION OF RESISTANCE TO SOGGY BREAKDOWN

If sugar content may be taken as a measure of stored reserve material in the apple, and nitrogen as a measure of catabolic activity, then the proportion of sugar to nitrogen may be indicative of resistance to breakdown or the storage capacity of the fruit. Haynes and Archbold (8) have suggested a relation between the ratio of sugar to nitrogen content of the apple and the length of storage life. The differences noted in the resistance to soggy breakdown between apples from unfertilized and fertilized trees, and between the fruit stored in different seasons, suggest the comparison of sugar to nitrogen ratios in comparable samples. Such ratios measured in mg. of reducing sugar and sucrose per mg. of nitrogen, on samples taken on the harvest dates for the three seasons, are depicted in figures 1, 2, 3 and 4.

Considering the ratios of reducing sugar to the nitrogen fractions and to total nitrogen (Figs. 1 and 2), it will be noted that these ratios were all higher for the two dry years (1930 and 1931) as compared with 1929. The situation held for both varieties under both treatments (fertilized and unfertilized orchards) and suggests a reason for the consistent higher resistance to soggy breakdown in Grimes and Jonathan apples in 1930 and 1931. It appears from these data that the reducing sugar/non-colloidal
nitrogen ratio is a better indicator of resistance to soggy breakdown than the reducing sugar/colloidal nitrogen ratio, since in Grimes in 1929 (Fig. 1) the two values of the latter ratio for the two treatments were more nearly the same. Moreover, the differences in reducing sugar to colloidal nitrogen ratios between samples from the two orchards in 1930 and 1931 were less marked than the differences in reducing sugar/non-colloidal ratios.

It will be noted (Figs. 1 and 2) that reducing sugar/non-colloidal nitrogen values were consistently higher in the fruit from check trees.
Fig. 2. Diagram showing correlations between the reducing sugar to nitrogen ratios and soggy breakdown in Jonathan apples. (orchard B fruit) than in the fruit from fertilized trees (orchard A fruit). This status held between comparisons in both varieties and in each of the three years of study. These higher reducing sugar/non-colloidal nitrogen
ratios in orchard B fruit again suggest a reason for greater resistance to soggy breakdown, which is in agreement with storage results when breakdown differences were apparent in 1929. Although differences in soggy breakdown were not apparent in 1930 and 1931, it is suggested that some other undetermined factor or factors associated with warm, dry weather were operative in making the fruit more resistant to this disorder. Among such may be listed the alcohol insoluble residue/nitrogen ratio, the total sugar plus organic acids/nitrogen ratio and others.

The relationship between the sucrose/nitrogen ratio on the picking date and the resistance to soggy breakdown in storage is of interest in this connection. Haynes and Archbold (l. c.) have pointed out that the supply of sucrose may be a limiting factor in the storage of apples and that the sucrose/nitrogen ratio may be indicative of storage behavior. A graphic representation of the sucrose/non-colloidal nitrogen, sucrose/colloidal nitrogen and sucrose/total nitrogen ratios for Grimes and Jonathan fruit from the two orchards A and B is shown in figs. 3 and 4, respectively. It will be noted that there were no consistent differences between the sucrose/nitrogen ratios in samples of fruit of the different years. That is, the ratios of sucrose to the various forms of nitrogen for the two years

![Fig. 3. Diagram showing correlations between the sucrose to nitrogen ratios and soggy breakdown in Grimes apples.](image-url)
1930 and 1931, when the fruit was highly resistant to soggy breakdown, were not consistently higher than the corresponding ratios in 1929, when the fruit was susceptible to soggy breakdown.

In a similar manner, in the comparison between the sucrose/nitrogen ratios of the fruit from the two orchards in the same years there also is no consistent relationship between these ratios and the percentage of soggy breakdown. The sucrose/non-colloidal nitrogen, sucrose/colloidal nitrogen and sucrose/total nitrogen ratios, as determined on the date of picking, therefore do not appear to have been indicative of the relative susceptibility of these apples to soggy breakdown. An explanation for the inconsistency of the sucrose/nitrogen ratio as an index of resistance to soggy breakdown in apples probably lies in the variability in sucrose

Fig. 4. Diagram showing correlations between the sucrose to nitrogen ratios and soggy breakdown in Jonathan apples.
content of individual apples because of a rapidly changing starch/sucrose ratio at picking time and during the latter part of the ripening period. Starch content decreases in fruit on the tree during this period and there is a corresponding increase in sucrose content. Reducing sugars change less than sucrose. The data in table 7 show that orchard A Grimes in 1929 increased over 42 per cent in sucrose content during the last 10 days on the tree, while reducing sugars increased only 13.5 per cent during the same interval. It appears, therefore, that sucrose content is more variable than reducing sugar in apples while ripening and this may be an explanation of the variability noted in the sucrose/nitrogen ratios.

ALCOHOL INSOLUBLE RESIDUE

Alcohol insoluble residue material was determined incidental to obtaining the analyses of colloidal nitrogen. It consisted of the tissue remaining after extraction with 80 per cent alcohol and included the insoluble materials starch, pectin, cellulose and other substances. Residues were determined on duplicate samples and close agreement was obtained in all cases.

The changes in residue values for Grimes fruit stored in 1929 are shown in tables 12 and 13. With samples stored immediately (table 12)

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>30°-31° F.</th>
<th>35°-36° F.</th>
<th>48°-50° F.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
<td>Check</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>4.120</td>
<td>4.534</td>
<td>4.120</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>3.148</td>
<td>3.278</td>
<td>3.053</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>2.829</td>
<td>3.008</td>
<td>2.866</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>2.754</td>
<td>2.723</td>
<td>2.691</td>
</tr>
<tr>
<td>March 15</td>
<td>2.670</td>
<td>2.646</td>
<td>2.695</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of sampling</th>
<th>30°-31° F.</th>
<th>35°-36° F.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Check</td>
<td>Plus N.</td>
</tr>
<tr>
<td>Sept. 26</td>
<td>4.120</td>
<td>4.534</td>
</tr>
<tr>
<td>Oct. 17*</td>
<td>3.615</td>
<td>3.744</td>
</tr>
<tr>
<td>Nov. 6</td>
<td>2.937</td>
<td>2.762</td>
</tr>
<tr>
<td>Dec. 1</td>
<td>2.751</td>
<td>2.607</td>
</tr>
<tr>
<td>Jan. 6</td>
<td>2.622</td>
<td>2.548</td>
</tr>
<tr>
<td>March 15</td>
<td>2.603</td>
<td>2.519</td>
</tr>
</tbody>
</table>

*Storage date.
the residues of plus nitrogen fruit remained higher with the 30-31° F. treatment until December 1; with the 35-36° F. treatment until November 6; with the 48-50° F. treatment only until a short time after storing, which was before the second analysis on October 17. With samples given deferred storage treatment (table 13) the residues of plus nitrogen fruit remained higher with the 30-31° F. and 35-36° F. treatments only until the date of storage (October 17).

It has been pointed out that sucrose content in the plus nitrogen Grimes in 1929 increased over three times as fast the last 10 days on the tree as check Grimes. It is suggested, therefore, that the higher residue of plus nitrogen fruit on the date of picking was due chiefly to the presence of more starch, probably as a consequence of slightly less maturity. Archbold (3) found the loss in alcohol insoluble residue on the tree in August to be due almost entirely to hydrolysis of starch. It seems reasonable to conclude, therefore, that after starch hydrolysis was complete in storage the subsequent higher residue content in the unfertilized fruit was the result of greater cell wall development, coincident with a higher degree of differentiation of wall tissues in the growth of the fruit. Since a greater quantity of cell wall material indicates more storage reserves in the form of pectic constituents and alcohol insoluble acid hydrolizable materials, this offers an explanation for premature breakdown in apples from nitrogen fertilized trees. It has been shown that more soggy breakdown occurred in fertilized fruit in 1929 than in check fruit, but that little difference in breakdown occurred between these treatments in 1930 and 1931. However, in seasons when fruit growth and ripening take place under normal seasonal conditions, it appears that the alcohol insoluble residue of apples can be taken as one measure of storage capacity.

SUMMARY

1. Sodium nitrate was applied, in various quantities and in different years in a high producing orchard, to Grimes and Jonathan trees. Samples of fruit from the nitrate treatments and from an adjacent untreated orchard were analyzed for nitrogen and sugar content at various intervals before picking and after storing throughout two seasons.

2. Sodium nitrate fertilizer appears to have greatly increased the susceptibility of Grimes and Jonathan apples to soggy breakdown in 1929, a year having about a normal growing season. In 1930 and 1931 little soggy breakdown occurred and no significant differences in the susceptibility of fruit taken from the plus nitrogen trees and minus nitrogen trees in the same orchard, or from the untreated trees in another orchard, were apparent.

3. Within the same orchard, nitrate applications consistently increased the nitrogen content of the fruit in each of the three years of this investigation. These increases were principally in the non-colloidal fraction. Within different orchards, the nitrated orchard produced apples higher in nitrogen content during the two dry years (1930 and 1931), but in the wet year (1929) the differences between the fruit from the two orchards were not significant. During the last two seasons, when nitrogen content varied significantly, little or no breakdown appeared in the
fruit. Therefore, there was no correlation between the nitrogen content of the fruit when it was picked and soggy breakdown susceptibility.

4. Non-colloidal nitrogen decreased in apples in storage. This decrease was more pronounced at the higher temperature employed (48-50° F.) than at cold storage temperatures. The results indicate that non-colloidal nitrogen in apples was modified more by storage temperature than the colloidal form. Under deferred storage at 48-50° F. there was a greater and more rapid loss of non-colloidal nitrogen in fruit from the unfertilized trees than in fruit from the nitrated trees. A higher non-colloidal nitrogen level in the plus nitrogen samples persisted throughout five and one-half months in storage.

5. Nitrate fertilizers appeared to increase the nitrogen content of the fruit harvested the same year of application, as well as that of fruit harvested the second year after application. However, fruit from trees which had not received nitrate fertilizer for three years showed no increased nitrogen content as a result of the treatment. The increased nitrogen content did not make the fruit more susceptible to soggy breakdown.

6. The results on sugar analyses, although not always consistent, showed in general that nitrate fertilizer slightly reduced the sugar content of the fruit. The data indicate that the sugar content of the fruit, when it is placed in storage could not be taken as a measure of breakdown susceptibility. The most marked changes during storage were in sucrose content; in a few instances sucrose reached a very low level in fruit from the nitrated trees. This was especially true in the case of fruit stored at the higher temperatures (48-50° F.). During the storage period reducing-sugar values indicated more stability than sucrose values.

7. Ratios of sugar to nitrogen indicated higher values of reducing sugar to the nitrogen fractions for the two dry years (1930 and 1931) as compared with 1929. This situation was consistent for both Grimes and Jonathan varieties, and for fruit from either the nitrated or check orchard. This result suggests a reason for the consistently higher resistance to soggy breakdown in 1930 and 1931, when little occurred. The reducing sugar/non-colloidal nitrogen ratio appeared to be a better indicator of resistance to soggy breakdown than the reducing sugar/colloidal nitrogen ratio.

8. Reducing sugar/non-colloidal nitrogen values were consistently higher in fruit from the unfertilized orchard than that from the fertilized orchard. The latter situation held for both varieties and for each of the three seasons and suggests a reason for greater resistance to soggy breakdown in fruit from the check orchard.

9. Ratios of sucrose to either of the nitrogen fractions determined, or to total nitrogen, taken on the picking date could not be correlated with susceptibility to soggy breakdown.

10. Alcohol insoluble residues from fertilized samples remained higher than those from check samples only during the forepart of the storage season. During the latter part of the storage period, after complete starch hydrolysis, the residues from the check samples were greater. The
exchange from higher residue values to lower, and vice versa, took place early or late, depending upon storage temperature. The consistently higher alcohol insoluble residue (after complete starch hydrolysis) in fruit from check trees suggests more cell wall differentiation in this fruit, and suggests one other reason for better storage capacity.

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15. Palmer, R. C.  


17. ---  

18. Thomas, Walter  

19. Weinberger, J. H.  
THE RELATION OF THE SIZE OF THE INFECTIVE DOSE TO
NUMBER OF OOCYSTS ELIMINATED, DURATION OF INFECTION,
AND IMMUNITY IN EIMERIA MIYAIRII OHIRA INFECTIONS
IN THE WHITE RAT

PHOEBE R. HALL

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To the author's knowledge, previous investigators have not deter­
mined (1) the number of oocysts eliminated following the feeding of vari­
ous sized doses of viable sporulated oocysts of a coccidium of the genus
Eimeria, (2) whether the length of time during which oocysts were passed
in the feces varied with the size of the infective dose, (3) the relative
amount of immunity produced by small infective doses of varying size,
and the minimum number of sporulated oocysts required to produce total
immunity and (4) the pathological effects of different sized doses, such
as effects on the mean daily weights of the hosts. The present study was
designed to make such determinations.

Rats used in these experiments were Wistar A rats (excepting pos­
sibly one litter used in the series infected with one oocyst per rat), and
were offspring of closely related parents. By using rats of such parentage,
all experimental animals had a similar genetical constitution and conse­
quently differences in yields due to hereditary differences in susceptibility
of the host were avoided as much as possible. It was noted that even then
there appeared litters which as a group out-yielded other litters which had
received a duplicate treatment. From the time of birth until approxi­
mately four weeks later, each litter of young rats was kept in a breeding
cage, the bottom of which was covered with a carpet of dry shavings.

The young rats, after being weaned, were fed a modification of the
Steenbock growth ration, green food at intervals of three or four days,
and milk and water ad libitum. The ration used was as follows:

Yellow corn meal .......... 76.0 lbs. Ground alfalfa ................. 2.0 lbs.
Linseed oilmeal .......... 16.0 " NaCl .......................... 0.5 "
Commercial casein ........ 5.0 " CaCO₃ .......................... 0.5 "
Dried buttermilk .......... 12.0 lbs.

At the age of five weeks, the rats were put on experiment. At this
time each animal was placed individually into a specially made cage of

*Taken from a thesis submitted to the faculty of the Graduate College, Iowa State
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*The writer wishes to take this opportunity to express her deep appreciation to
Dr. Elery R. Becker for his assistance in outlining the problem and enthusiastic direc­
tion throughout the progress of the investigation.

The financial support of the experiments came from the Rockefeller Fluid Re­
search Fund at Iowa State College and the Industrial Science Research Fund at the
same college.

[115]
three mesh hardware cloth fitted over a 9" x 12" aluminum or enamel pan. The rats were weighed daily after being put on the experiment. The diet, thereafter, consisted of only the grain mixture and water. Previous to the time of the experimental feeding of oocysts, none of the rats had an infection. Until then, all young growing rats were examined at regular intervals of three or four days, and had there been accidental infection, it would have been detected.

The parasite used in the experiment was a selected strain of *Eimeria miyairii*. This strain was selected and inbred in the following manner. With a micropipette a single sporulated oocyst was secured from a field under low (100X) power of the microscope. Then by placing the pipette into the mouth of a partially anesthetised rat this oocyst was administered to its host. The oocysts which developed from this one were collected in a two per cent solution of potassium dichromate, and from this culture another single oocyst was isolated and similarly administered to another rat. This process was repeated until the parasite had been inbred for the third generation. It was believed at this time that whatever oocysts might be selected by chance from this highly inbred strain would be representative of a fairly homogeneous population.

1. NUMBER OF OOCYSTS PRODUCED BY DIFFERENT SIZED INFECTIVE DOSES

The number of oocysts eliminated following a series of five infective doses of 1,500 oocysts each was reported in 1932 by Becker and Hall as falling between 14,100,000 and 169,220,000. No study, however, has been made to determine the yield from a single dose of a known number of sporulated oocysts. It was felt that information concerning the normal number of oocysts produced by any known number of parasites would be of value in the study of effects produced during an infection, and also would determine the optimum dose for experimental purposes with the rat as the host. It was with these motives in view that the author has attempted to determine the relationship which exists between the number of sporulated oocysts fed to an animal and the number of parasites passed in the feces of the infected animal during the period of oocyst elimination.

In the determination of the yield of oocysts from a given number of parasites, the infective doses contained the following number of organisms, respectively: one, four, ten, fifty, one hundred, fifteen hundred, fifteen thousand, and fifteen hundred thousand. Of thirty-three attempts to infect rats with a single parasite, only twenty-two trials proved to be successful. From these figures it was concluded that only two-thirds of the number of oocysts given to any one rat succeeded in completing the sexual phase of their life history in the intestine. This assumption was thereafter respected in the calibration of all cultures used for infective purposes. The small doses of one, four and ten oocysts were isolated with a micropipette by selecting the individual oocysts from a field under low power of the microscope. The actual selection consisted of one, six and fifteen oocysts, respectively.

In giving doses of fifty oocysts or more, the dilution method was used in measuring the dose. In each of the latter cases, the culture was so calibrated that approximately the desired number of parasites was suspended in one cubic centimeter of liquid.
The rats were etherized and given the cubic centimeter of the suspension containing the oocysts from a syringe through a catheter. For each group of rats infected with the different sized doses, a number of rats of the same litter were kept for controls.

On the sixth day after the date of infection the pans under the experimental animals were thoroughly cleaned, and to each was added three hundred cubic centimeters disinfectant. The disinfectant used varied from twenty-five hundredths per cent to five-tenths per cent solution of “Kreso,” the approximate chemical composition of which is cresol, two and five-tenths per cent; soap (dry), twenty-three per cent; and inert ingredients, seventy-four and five-tenths per cent.

The fecal pellets were allowed to collect in the pans until elimination of oocysts ceased. The pellets were then broken up by mashing with a miniature stamper consisting of a solid one-inch rubber stopper into one end of which was inserted a glass stirring rod one-fourth inch in diameter and five inches in length. The suspension of fecal matter in the disinfectant was transferred from the collecting pan to a heavy glass container in which it was thoroughly homogenized by an electric mixer. The mixture was diluted with water, the exact dilution depending upon the amount of solids in the feces collected. After the material was again thoroughly agitated, the larger solid particles were removed from a small sample by straining through wire screens. This process was done in a fashion which averted a probable straining out of oocysts by solid particles accumulated on the filter. Screens through which the material was strained were of two sizes; namely, sixteen and twenty-four mesh, respectively. Following another thorough mixing a small amount of the suspension was immediately transferred to a haemocytometer three-tenths millimeter square and one-tenth millimeter deep. For determining the yield from a single parasite, the counting chamber, or haemocytometer was filled twenty times. For the larger ones, however, only eight or ten counts were made, the number of counts depending on the extent of variation of the first eight. Then from the known number of oocysts in either eighteen, nine or seven and two-tenths cubic millimeters, the total yield was calculated by multiplying the number of parasites counted by the ratio of the total volume of diluted material to the volume containing the oocysts counted.

Counts of the oocysts eliminated by the individual rats on the experiment showed that the greater the infective dose of parasites the greater was the yield of oocysts, but the ratio was not by any means constant. In table 1 are recorded the mean yields from the different sized infective doses, and the number of oocysts produced per oocyst fed in the various infections. When only one oocyst was fed there was a mean yield of approximated 62,000, while following an infective dose of four viable sporulated oocysts 2,182,500 oocysts per oocyst fed were produced. Why the yield per oocyst given for four oocysts should be thirty-five times that of the one oocyst infection is at present unexplainable. It might be due to increased chances for the union of the microgametes and the macrogametes in the case of the larger infective dose. It can be readily seen that an increase in number of parasites fed would cause the liberation of merozoites in larger quantities, which in turn would develop into more gamete-

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*Records of individual rats are printed in the original thesis No. 295 on file in the Iowa State College Library, Ames, Iowa.*
producing cells. The larger the number of gametes, the greater their proximity, and consequently the greater the likelihood of the microgametes encountering the macrogametes. This, however, does not explain why the oocyst production per oocyst fed should again gradually decrease from 2,084,000 when ten oocysts were fed to 1,000 when fifteen hundred thousand were fed. The latter may be an immunity phenomenon, or the result of depletion of suitable host cells for colonizing. The nature of the immunity produced and the exact time required for its production are not known, but here again the marked comparative decrease in yield per oocyst in the case of the higher dosage might perhaps be due to a partial

**TABLE 1. Number of oocysts produced by different sized infective doses**

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>Number of oocysts in infective dose</th>
<th>Mean yield/(10)^4</th>
<th>Standard deviation/(10)^4</th>
<th>Oocysts produced per oocyst fed/(10)^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>1</td>
<td>6.24 ± 0.029</td>
<td>0.21</td>
<td>6.24</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>873 ± 40</td>
<td>260</td>
<td>218.25</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>2,084 ± 155</td>
<td>892</td>
<td>208.40</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>8,225 ± 502</td>
<td>2,977</td>
<td>164.70</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>15,445 ± 1,377</td>
<td>7,360</td>
<td>154.45</td>
</tr>
<tr>
<td>11</td>
<td>1,500</td>
<td>28,520 ± 1,323</td>
<td>6,472</td>
<td>19.22</td>
</tr>
<tr>
<td>7</td>
<td>15,000</td>
<td>38,254 ± 2,336</td>
<td>9,147</td>
<td>2.61</td>
</tr>
<tr>
<td>1</td>
<td>1,500,000</td>
<td>155,000</td>
<td>.10</td>
<td></td>
</tr>
</tbody>
</table>

immunization by the larger numbers of merzoites which prevents the development of a proportionate number of gametocytes, and hence gametes. There remains also the possibility that there is during the infection a temporary depletion of invadable epithelial cells, as suggested above. It seems, however, that if this were the case, the total oocyst yield would be constant after a maximum was once reached.

2. DURATION OF INFECTION

It was ascertained by daily fecal examination that all rats on the various experiments began to eliminate oocysts either on the seventh or the eighth day after infection. According to Andrews (1) the time required for the endogenous cycle to become completed is the prepatent period. It will be seen from table 2 that the first appearance of oocysts was on the seventh day in the majority of cases. Even though oocysts were not found until the eighth day, all fecal matter eliminated on the seventh day was saved. This precaution was taken for fear there were a few oocysts eliminated by all rats on the seventh day, but because of random sampling the fecal smears examined did not reveal their presence. In no case was the oocyst yield at its maximum intensity before the ninth or tenth day after the administering of the viable oocysts. Table 2 also gives the duration of the period of oocyst elimination in days. It will there be noted that this period varied from three to five days, inclusive. The data show that the mean patent period varies from 4.2 days following the feeding of small infective doses to 5 days following an infective dose of 1,500,000 oocysts. The author questions whether there is actually an increase in the
length of the period of oocyst elimination corresponding to the increase in number of oocysts fed. The seeming difference may be merely due to the oocysts being present in larger numbers sooner after elimination begins and for a longer period after the time of maximum yield has been reached. With an increase in the number of oocysts present, there would be a corresponding increase in the chances for their being seen in the sample of feces examined.

**TABLE 2. Effect of size of dose on duration of infection**

<table>
<thead>
<tr>
<th>Number of oocysts in infective dose</th>
<th>Number of rats on experiment</th>
<th>Prepatent period</th>
<th>Patent period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>7 days</td>
<td>8 days</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>1,500</td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>15,000*</td>
<td>7</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>1,500,000*</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*The data given here apply only to rats which survived throughout the infection.

3. IMMUNIZING EFFECTS OF DIFFERENT SIZED DOSES

It was reported by Becker and Hall (1932) that the feeding of 1,500 viable sporulated oocysts on each of five consecutive days produced a total immunity. It was realized that five feedings of this dose were more than were required for the production of total immunity. It was suggested by Johnson in 1927 (5) and again by Tyzzer in 1929 (6) that a light infection prevented a heavy subsequent reinfection, but no quantitative study of immunity produced by small infective doses had been made.

To determine whether the rats which had been infected with a given number of oocysts were either partially or entirely immunized, the previously infected experimental animals together with the controls were given fifteen hundred oocysts on each of five consecutive days, as it was known that this dose would produce total immunity in the control animals. To avert physiological interference between the two infections the attempts at reinfection were not made until the cessation of elimination of oocysts produced by the first infection. The same treatment was given to rats during the second infection as during the first, and the previously discussed technique employed in making determinations of oocyst yields was again used. The yield of oocysts from previously infected rats compared with that of the controls was taken as an index to the amount of immunity produced by the various sized infective doses.

Efforts to determine the minimum dose which would cause an appreciable loss in susceptibility of the host revealed that an infection as small
as that produced by four oocysts produces a degree of immunity worthy of note. The yields from the multiple infections following a single infection of 4 oocysts ranged from \(600 \times (10)^4\) to \(2,292 \times (10)^4\), while the yield from the controls for that same group ranged from \(2,292 \times (10)^4\) to \(26,187 \times (10)^4\).

Testing this difference by the pooled sum of squares method, recommended by Fisher (4) for small samples, gives a value of 3.77 for \(t\). According to Fisher a value of only 2.724 for \(t\) is sufficient to denote differences which are highly significant.

These experiments were repeated using 10, 50, 100, 1,500 and 15,000 oocysts, respectively, the significance of the results of which were statistically tested and summarized in table 3.

**Table 3. Significance of susceptibility lost on account of previous infection**

<table>
<thead>
<tr>
<th>Oocysts in first infection</th>
<th>No.</th>
<th>Mean yield ((10)^4)</th>
<th>Standard deviation ((10)^4)</th>
<th>Lowest yield ((10)^4)</th>
<th>Highest yield ((10)^4)</th>
<th>Value of (t)</th>
<th>Value of (t) above which differences are highly significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>19</td>
<td>7,840 ± 1,055</td>
<td>6,805</td>
<td>680</td>
<td>28,368</td>
<td>3.77</td>
<td>2.724</td>
</tr>
<tr>
<td>Controls</td>
<td>18</td>
<td>16,525 ± 1,172</td>
<td>7,202</td>
<td>2,292</td>
<td>26,187</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>4,069 ± 680</td>
<td>3,902</td>
<td>221</td>
<td>11,259</td>
<td>4.988</td>
<td>2.763</td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>17,818 ± 1,592</td>
<td>9,135</td>
<td>5,618</td>
<td>33,913</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>15</td>
<td>1,447 ± 108</td>
<td>625</td>
<td>425</td>
<td>8,300</td>
<td>4.618</td>
<td>2.763</td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>22,379 ± 3,035</td>
<td>9,200</td>
<td>7,978</td>
<td>36,650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>223 ± 26</td>
<td>144</td>
<td>14</td>
<td>569</td>
<td>4.34</td>
<td>2.807</td>
</tr>
<tr>
<td>Controls</td>
<td>11</td>
<td>20,500 ± 3,817</td>
<td>18,730</td>
<td>5,597</td>
<td>58,944</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1500</td>
<td>11</td>
<td>636 ± 410</td>
<td>2,013</td>
<td>0</td>
<td>7</td>
<td>7.757</td>
<td>2.845</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>11,259 ± 1,020</td>
<td>4,780</td>
<td>5,069</td>
<td>20,847</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15000</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>14</td>
<td>10,128 ± 685</td>
<td>3,210</td>
<td>5,221</td>
<td>16,100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 shows that there is a conclusive evidence that a single infection produces an appreciable amount of resistance in the body of the host. The question arises as to how much relative susceptibility is lost by the host in infections originating from known numbers of parasites. Furthermore, from the standpoint of the host, what is the optimum dosage for producing a high degree immunity for protection against subsequent reinfection?

In order to get the loss of susceptibility due to the different sized doses in comparable terms, the author has attempted to estimate the percentage of total immunity acquired during the first infection by assuming the controls for each group to be 100 per cent susceptible. The
percentage of immunity acquired was then figured by obtaining the following ratio:

\[
\frac{M \times N \times 100}{Mc \times Nc}
\]

Where \( M \) and \( N \) are the mean of yield and number of experimental animals, and \( Mc \) and \( Nc \) are the mean of yield and number of controls, respectively.

Figuring the percentages in this way, infective doses as small as 4 oocysts were found to produce as much as 53.02 per cent immunity, while a single dose of 1,500 oocysts caused a loss of 99.995 per cent of total susceptibility. None of the infections due to 1,500 oocysts or less seemed to produce any severe clinical symptoms.

Table 4 gives the fraction of total immunity gained by different sized doses. It is of interest to note that while a dose of 1,500 oocysts produced 99.995 per cent total immunity, doses of 50 and 100 produced almost as much—90 and 98 per cent, respectively. There were in the first group eleven experimental animals, ten of which were completely immunized after the single infective dose of 1,500 oocysts.

**Table 4. Effect of previous infection on oocyst yield after standard immunizing dose (1,500 oocysts daily for 5 days)**

<table>
<thead>
<tr>
<th>Number of oocysts in infective dose</th>
<th>Mean yield from previous infection</th>
<th>Oocysts produced per oocyst fed to experimentals</th>
<th>Oocysts produced per oocyst fed to controls</th>
<th>Percentage of total immunity produced by first infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>( 873 \times (10)^{1} )</td>
<td>10,452</td>
<td>22,033</td>
<td>53.02</td>
</tr>
<tr>
<td>10</td>
<td>( 2,084 \times (10)^{1} )</td>
<td>5,429</td>
<td>23,757</td>
<td>77.15</td>
</tr>
<tr>
<td>50</td>
<td>( 8,235 \times (10)^{1} )</td>
<td>1,930</td>
<td>20,000</td>
<td>90.35</td>
</tr>
<tr>
<td>100</td>
<td>( 15,445 \times (10)^{1} )</td>
<td>233</td>
<td>27,300</td>
<td>98.03</td>
</tr>
<tr>
<td>1,500</td>
<td>( 28,830 \times (10)^{1} )</td>
<td>8</td>
<td>15,012</td>
<td>99.995</td>
</tr>
<tr>
<td>15,000</td>
<td>( 38,254 \times (10)^{1} )</td>
<td>0</td>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

4. WEIGHT CHANGES, PATHOLOGICAL EFFECTS AND LETHAL DOSAGE

No apparent clinical symptoms were evident in rats receiving an infective dose of one or four oocysts. In an infection resulting from ten to fifteen hundred oocysts, the most noticeable symptoms were general sluggishness and loss of responsiveness to raps on the cage on about the seventh and eighth days after the date of infection. Not until the single infective dose was increased to 15,000 viable oocysts did the experimental animals gain weight at an apparently less rapid rate than did normal rats during the same period.

In the lighter infections there were days on which there was no appreciable gain, but corresponding weight changes were usually observed in the control animals. The author believes, therefore, that to place too much emphasis on the gain or loss of weight due to the lighter infections
would be misleading since some of the differences might chance to be due merely to physiological coincidents rather than to pathological conditions.

Following an infection with a dose as large as 15,000 oocysts, however, there was during the infection a percentage gain over the initial weight which appears to be significantly smaller than that of the controls during the same period. The experimental animals in this group weighed an average of 77 grams at the beginning of the infection, and gained during the experiment twenty-three per cent of their initial weight; while the controls weighed only 70 grams to begin with, and before the end of the experiment had increased in weight forty-four per cent. This observation is still more impressive when it is taken into consideration that forty-six per cent of the animals infected with this sized dose died on the eighth day after the date of infection, and that the animals here considered represent the more resistant survivors.

It was found that by feeding 15,000 oocysts there was a forty-six per cent mortality on the eighth day of the infection, whereas with a larger dose of 1,500,000 oocysts, ninety-two per cent of the fourteen rats died within thirty-six hours after being infected. All rats in either case developed a severe diarrhea before death, and a post mortem examination showed the small intestine to be markedly hemorrhagic.

**SUMMARY AND CONCLUSIONS**

1. The mean yields of the oocysts of *Eimeria miyairii* from single infective doses of different sizes were as follows:

<table>
<thead>
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<td>1,500,000 &quot;</td>
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2. The prepatent period for a single infective dose ranging from one to 1,500,000 sporulated oocysts is approximately seven days. The patent period for the same varies from three to five days, and in the majority of cases is either four or five days. Its length seems to be independent of the size of the infective dose.

3. Different sized infective doses are in no way reliably indicative of a predictable gain or loss in weight during the infection. There is naturally a big variation in the daily gains of weight among rats, but this variation is not in general affected by the coccidial infections produced by from one to 1,500 sporulated oocysts. Single infective doses of 15,000 oocysts, however, cause an increase in weight during the infection significantly less than that of the controls during the same period of time. This difference is most manifest during the first nine days of the infection.

4. Single infective doses as small as four viable sporulated oocysts cause a rat to lose approximately fifty per cent of its natural susceptibility;
### Table 5. Effect of different sized infective doses on mean daily gain in weight in grams

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Initial weight: 100

Total gain during infection: 39

Percentage gain over initial weight: 39
ten oocysts, approximately seventy-five per cent; fifty oocysts, approximately ninety per cent; one hundred oocysts, approximately ninety-eight per cent; and fifteen hundred, approximately one hundred per cent.

5. Single infective doses of 15,000 viable oocysts prove fatal on the eighth day of the infection in approximately fifty per cent of the cases, while after the much larger dose of 1,500,000 sporulated oocysts there is approximately a ninety-five per cent mortality within thirty-six hours after the time of inoculation.

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ORGANISMS PRODUCING A POTATO ODOR IN MILK

H. C. OLSON AND B. W. HAMMER

From the Laboratories of Dairy Bacteriology, Dairy Industry Section, Iowa Agricultural Experiment Station

Accepted for publication September 17, 1934

A potato odor is occasionally encountered in milk and cream. There appear to be at least three possible causes of this condition: (a) holding unprotected milk or cream where there is the odor of potatoes, (b) feeding potatoes to the cows producing the material and (c) the action of microorganisms. Two types of bacteria causing a potato odor in dairy products have been isolated at the Iowa Agricultural Experiment Station. The work herein reported deals with the identification and comparison of these two types.

PSEUDOMONAS GRAVEOLENS

Recently an outbreak of a potato odor in milk from a certain farm over a period of about two weeks was reported by an Iowa milk plant. Inquiry by the plant management revealed that the milk was not exposed to the odor of potatoes and that potatoes were not being fed to the producing animals. A sample of the milk was plated, using beef infusion agar and incubating at 21°C; after about 15 hours considerable numbers of colonies were evident, and the plates had an odor definitely suggestive of potatoes. On picking cultures into litmus milk, a number of them, all of which seemed to be of the same type, quickly produced a potato odor at 21°C. The odor, which duplicated that in the sample of milk, was very conspicuous and so definite that it was described as resembling potatoes by various persons whose attention was called to it. When there was a number of milk or agar cultures of the organism together, the odor could often be detected on entering the room where they were being held.

A study of the morphologic, cultural and bio-chemical characters of the organism isolated definitely identified it as Pseudomonas graveolens, which was described by Levine and Anderson (4) following its isolation from musty eggs. A comparison of the organism with a culture of Ps. graveolens used in the preparation of the original description showed that the two were the same, although they differed in the intensity of the potato odor produced. Cultures of the organism isolated were grown in various media and submitted to Levine and Anderson. They considered the potato odor identical with the odor which they had termed musty. The odor-producing characteristic of their culture had apparently been partially lost during numerous transfers on laboratory media.

Ps. graveolens has a number of characteristics that should be considered in connection with its ability to cause odor defects in dairy products. It fails to grow at 37°C, and when plates poured with the defective milk, from which Ps. graveolens was isolated, were incubated at this tempera-


2Babcock (1) reported that feeding cows potatoes one hour before milking resulted in only slight abnormal flavors and odors in the milk.
ture the organism did not develop. In a flask of aseptic milk inoculated with the organism and held at about 8° C., the count increased from 12,900 per ml. immediately after inoculation to 5,500,000 per ml., after 96 hours, when the potato odor was very evident. These results indicate that ordinary cooling will not prevent the growth of *Ps. graveolens* in milk. The ability of the organism to grow rapidly at ordinary temperatures may make it a serious contaminant on farms where thorough cleaning and sterilizing of the utensils are not practiced.

On extended holding, cultures of *Ps.graveolens* often developed an odor more like that of turnips than of potatoes.

In order to determine the effects of pasteurization on the potato odor, a small lot of pasteurized milk was inoculated with *Ps. graveolens* and held at 21° C. until a definite potato odor had developed. Half of the lot was then re-pasteurized at 62.8° C. for 30 minutes and compared with the half not pasteurized after inoculation. The comparison showed that the intensity of the odor was not decreased by the pasteurization.

**Pseudomonas mucidolens**

In the studies carried out at the Iowa Agricultural Experiment Station some years ago on the changes in flavor and odor that occur in cream held at relatively low temperatures, a sample of cream was encountered that had developed an odor resembling potatoes. An organism that produced a potato odor in milk or cream was easily isolated by plating on beef infusion agar and incubating the plates at 21° C. The odor was very conspicuous and readily recognized. The organism was identified as *Pseudomonas mucidolens*, which was described by Levine and Anderson (4) following its isolation from musty eggs. A comparison of the culture isolated with a culture of *Ps. mucidolens* used in the preparation of the original description showed the two agreed in their general characters, although the culture isolated from cream reduced nitrates more slowly and less extensively, produced hydrogen sulfide more slowly, and hydrolyzed fat more rapidly than the other culture. Moreover, the culture isolated from cream frequently showed several flagella at one pole, while the cultures secured by Levine and Anderson showed a single flagellum.

**Additional Observations on Ps. graveolens and Ps. mucidolens**

From the studies carried out, a number of characters of importance can be added to the published descriptions of *Ps. graveolens* and *Ps. mucidolens*. The former organism did not attack various fats, while the latter hydrolyzed various fats as shown by Nile-blue sulphate and also by the production of rancidity in butter made from cream to which the organism had been added. In some instances *Ps. mucidolens* produced rancidity in milk and cream along with the potato odor; rancidity was very slight in milk, however. *Ps. graveolens* showed a single flagellum, as did the culture of *Ps. mucidolens* used in the preparation of the original description, while the culture of *Ps. mucidolens* isolated from cream frequently showed several flagella at one pole. Both types grew well in Dunham's peptone solution, but neither showed growth in Uschinsky's solution.

In bouillons (0.5 per cent peptone and 0.3 per cent beef extract) containing one per cent of various fermentable materials, the following reac-

---

*The isolation was made by M. A. Collins.*
Organisms producing a potato odor in milk

Tions were secured with Ps. graveolens and Ps. mucidolens at 21° C., bromcresol purple being used as the indicator. Gas was not produced by either organism. Both types formed acid in two to four days from arabinose, dextrose and galactose, but not from sucrose, maltose, lactose, raffinose, dextrin, starch, inulin, glycerol, dulcitol, mannitol, sorbitol, adonitol, inositol or salicin. From levulose Ps. graveolens formed a slight acid reaction after 18 days and Ps. mucidolens after 8 days; from rhamnose both types produced a slight acid reaction after 18 days. Using a peptone-dipotassium phosphate medium, with Andrade's indicator, Levine and Anderson (4) secured reactions differing somewhat from the above.

Levine and Anderson (4) found that in litmus milk Ps. graveolens showed "reduction and coagulation after two weeks with an acid ring on the surface." With the cultures of Ps. graveolens studied, including a culture from Levine and Anderson, the reaction in litmus milk was neutral or slightly alkaline at first, later becoming definitely acid and reduced at the bottom; some of the cultures eventually coagulated the milk.

Other organisms producing a potato odor in milk or cream

A lot of cream having a potato odor was studied by Brannon (2), who isolated an organism which reproduced the characteristic odor within 24 hours after inoculation into freshly separated cream held at 21.1° C. Inoculated cream incubated at 10.0°, 26.7° or 7.38° C. did not develop the odor. Brannon also mentioned having previously isolated an organism which produced a potato flavor and ropiness in cream; however, this organism soon lost its ability to produce the characteristic flavor.

In further observations on the organism producing a potato odor, Brannon (3) stated that, "If cream in which this organism has grown and produced the potato odor is churned into butter the potato odor persists. This organism has been inoculated into milk which later was converted into other dairy products and if the potato odor appeared in the milk it also appeared in the manufactured product." Brannon did not identify his organism with any of the known species or suggest a name for it.

Summary

Two organisms causing a potato odor in milk or cream have been identified as Pseudomonas graveolens and Pseudomonas mucidolens. In addition to the production of the potato odor, Ps. mucidolens also hydrolyzed fat actively; it produced rancidity in butter, and sometimes in milk and cream.

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## CONTENTS

Abstracts of Doctoral Theses

<table>
<thead>
<tr>
<th>Title</th>
<th>Author</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>The determination of the thermionic work function of nickel by a new method.</td>
<td>ROBERT M. BOWIE</td>
<td>131</td>
</tr>
<tr>
<td>The use of polymers from furfural in the fabrication of molded products.</td>
<td>RICHARD WEDGE BRUINS</td>
<td>133</td>
</tr>
<tr>
<td>Quantitative studies on the formation of xylose from pentosan-containing materials.</td>
<td>LOREN C. BRYNER</td>
<td>137</td>
</tr>
<tr>
<td>Condensation reactions of furfural and its derivatives.</td>
<td>NATHANIEL OGLESBY CALLOWAY</td>
<td>141</td>
</tr>
<tr>
<td>Bacteriological studies on butter showing surface taint.</td>
<td>HERBERT ANDREW DERBY</td>
<td>145</td>
</tr>
<tr>
<td>Studies on vitamins B and G in growth and lactation in the rat. (A) The effects of extracts of vitamins B and G. (B) The distribution of vitamin G.</td>
<td>JAMES FLOYD FEASTER</td>
<td>147</td>
</tr>
<tr>
<td>Some factors affecting the growth of Penicillium roqueforti in cheese.</td>
<td>NORMAN S. GOLDING</td>
<td>151</td>
</tr>
<tr>
<td>Causes of slow acid production in butter cultures.</td>
<td>L. A. HARRIMAN</td>
<td>155</td>
</tr>
<tr>
<td>Plastics from high pentosanocellulosic material.</td>
<td>WILLIAM DONALD HARRIS</td>
<td>159</td>
</tr>
<tr>
<td>A study of chlorine sterilizing compounds. I. Relationship between pH and oxidation potentials.</td>
<td>ARTHUR PAUL HELLWIG</td>
<td>163</td>
</tr>
<tr>
<td>The determination of the effect of manganese and sulfur on the malleabilization of white cast iron.</td>
<td>ARNOLD P. HOELSCHER</td>
<td>165</td>
</tr>
<tr>
<td>The role of inorganic substances and amino acids in the regeneration of hemoglobin in the rat.</td>
<td>HAVARD LAWRENCE KEIL</td>
<td>169</td>
</tr>
<tr>
<td>The biochemistry of the production of 2,3-butylen glycol by fermentation.</td>
<td>ANSON R. KENDALL</td>
<td>171</td>
</tr>
<tr>
<td>The effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk.</td>
<td>C. BRONSON LANE</td>
<td>173</td>
</tr>
<tr>
<td>Physiological and toxicological studies on insects.</td>
<td>EDWARD RAWSON MCGOVAN</td>
<td>177</td>
</tr>
<tr>
<td>A study of the Corynebacteria (Diphtheroids) associated with diseases of domestic animals.</td>
<td>IVAL ARTHUR MERCHANT</td>
<td>181</td>
</tr>
<tr>
<td>I. Some factors affecting the production of insulation board. II. The development of the commercial production of refrigeration board and pressboard.</td>
<td>THEODORE R. NAFFZIGER</td>
<td>183</td>
</tr>
<tr>
<td>Some of the physical-chemical properties of hog cholera virus. I. Filterability of hog cholera as affected by the hydrogen ion concentration. II. The migration of hog cholera virus when subjected to electrophoresis. III. Experiments on the attenuation of virus and the production of immunity to hog cholera.</td>
<td>LOUIS HAROLD SCHWANTE</td>
<td>187</td>
</tr>
<tr>
<td>The biochemistry of sluggish butyl-acetonic fermentations.</td>
<td>DONALD F. STARR</td>
<td>195</td>
</tr>
<tr>
<td>The butyl-acetonic fermentation of the sugars with special reference to xylose.</td>
<td>LELAND A. UNDERKOFER</td>
<td>197</td>
</tr>
</tbody>
</table>

NOTE: Complete copies of these theses can be consulted at the Library, Iowa State College, Ames, Iowa.
The production of alcohols by thermophilic fermentations. MATTHEW KERMIT VELDHUIS ......................................................... 201
The alkaline oxidation of lignin. ARTHUR WILLIAM WALDE .......... 205
Studies of the Escherichia-aerobacter group of bacteria in dairy products. MAURICE WADE YALE ....................................................... 209
Some quantitative studies of organometallic compounds. ERWIN A. ZOELLNER ........................................................................ 213
THE DETERMINATION OF THE THERMIonic WORK FUNCTION
OF NICKEL BY A NEW METHOD

ROBERT M. BOWIE

From the Department of Physics, Iowa State College

Accepted for publication August 1, 1934

The photoelectric work function of nickel has been determined a
number of times in the past (1, 2, 3, 4, 5, 6). Due to contaminated surface
conditions or poor vacuum technique, the earlier values were too low,
ranging from 3.6 volts to 4.5 volts. However, Glascoe (6) in 1931, using
Fowler’s (7) method of analysis and after a prolonged period of outgassing,
obtained the value of 5.01 ± 0.02 volts. The only previous thermionic
determination was made by Schlichter (8) in 1915. Because of poor vacu­
um technique he obtained the erroneous value of 2.77 volts.

It was the purpose of this work to redetermine the thermionic work
function of nickel by a new and more suitable method and to compare
the result so obtained with that of Glascoe. The development of the new
method was made necessary because the conventional method requires
a strip of wire of uniform cross-sectional area to be heated by an electric
current. During the prolonged outgassing process required, the uneven
evaporation of nickel would cause hot spots to develop which would in­
validate the results.

The method and the results have been given in a paper by Fox and
Bowie (9), the abstract of which is given in the following paragraph.

The metal sample was in the form of an approximate sphere and was
heated by electron bombardment from an auxiliary filament which was
disconnected when measurements were made. Electron emission from
the cooling sample charged a condenser, which, at predetermined times,
was discharged through a ballistic galvanometer. Temperatures were
determined by a Pt. Pt ± 10 per cent Rh thermocouple spot-welded to
the sample. The thermionic constants were obtained from the equation:

\[
\log_{10} \left( \frac{T^2}{SQ} \right) = \log_{10} \left( \frac{2.3}{aA} + Q \left( 1.988 \times 10^{-4} T \right) \right),
\]

where Q is the quantity of charge yet to flow upon cooling the sample from a given tempera­
ture to absolute zero, \(-S\) is the slope of the \(\log_{10} Q\) vs. \(X\) time curve. This equation is derived from Richardson’s, \(a\) is the area of the sample, \(A\)
is the constant in Richardson’s Equation, and \(T\) is the temperature of the
sample in degrees Kelvin. The values of the thermionic constants ob­
tained by applying this method to the case of thoroughly outgassed nickel
are found to be \(Q = 5.03 \pm 0.05\) volts and \(A = 1.38 \times 10^3\) amp/cm² deg.².

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6. Glasoe, G. N.  

7. Fowler, R. H.  

8. Schlichter, W.  

9. Fox, G. W., and R. M. Bowie  
THE USE OF POLYMERS FROM FURFURAL IN THE FABRICATION OF MOLDED PRODUCTS

RICHARD WEDGE BRUINS

From the Department of Chemical Engineering, Iowa State College
Accepted for publication August 1, 1934

The addition of sulphur or hydrochloric acid to a 20 per cent solution of furfurin in furfural produces a black, resinous body. This material is quite acidic, due to the acid used to produce the polymerization, and has a tendency toward shrinking and cracking. This plastic has been made the subject of a series of experiments in an endeavor to find some method by which the defects of the material could be corrected, and to adapt the plastic to the fabrication of molded products.

The treatment of an aqueous solution of furfural with hydrogen sulphide results in the formation of a colloidal solution of furfural polysulphide. Experiments were conducted to determine if the treatment of pure furfural with hydrogen sulphide could be used in producing a molding material. A black, hard, shiny resin was produced by bubbling hydrogen sulphide through furfural at 110° C. for one hour, and polymerizing this solution by adding 15 parts of concentrated hydrochloric acid to 100 parts of H₂S-furfural by volume. This material had a tensile strength of 800 pounds per square inch, but cracked shortly after removal from the mold. A mixture of furfurin solution and furfural treated with hydrogen sulphide was used. A mixture of 50 parts (by volume) of furfural treated with hydrogen sulphide for one hour, 30 parts of furfurin solution, and 20 parts concentrated hydrochloric acid resulted in a material having a tensile strength of 850 pounds per square inch. This compound also cracked after removal from the mold. The cracking can be prevented by coating the articles with paraffin, so as to prevent contact with the air.

Numerous compounds were tested to determine their ability to cause catalytic polymerization of the furfurin solution, and to discover if their addition would produce a material less susceptible to cracking. Of these compounds, sodium tungsatte and gallic acid produced a slightly more rapid solidification. The addition of small amounts (2 per cent by weight) of ferrous ammonium sulphate decreased the cracking tendency slightly, as did manganous and ammonium chlorides. Silicon tetrachloride was tested as a polymerizing agent. A hard, very brittle resin resulted when 8 parts of silicon tetrachloride was added to 100 parts of furfurin, by volume. The polymerizing effect of the silent electric discharge did not produce a solid material.

The introduction of anhydrous ammonia into furfural is accompanied by an increase in the specific gravity of the solution. This increase of specific gravity is of interest, since it permits a determination of the concentration of the furfurin solution by obtaining the specific gravity and reading the corresponding concentration from a suitable table.

1Original thesis submitted June, 1934.
The properties of many resins are improved by the addition of a plasticizer. Dibutyl phthalate was tested as a plasticizer. Due to the compound's immiscibility with the aqueous acid used, the phthalate separated from the mixture. Non-aqueous solutions of hydrochloric acid, such as an alcoholic solution, were tried. The resins produced using furfurin solution, dibutyl phthalate, and a solution of hydrochloric acid in butyl alcohol were hard, glossy, and had a lower density than the furfurin-solution-aqueous hydrochloric acid resins. A slight tendency to crack was observed, although in numerous instances samples were obtained which were free from cracking.

Acetone was substituted for butyl alcohol as a solvent for hydrochloric acid gas, with excellent results. The freedom from cracking of these materials was a quality which had not been observed in any other resin. Sulphuric acid was mixed with the acetone, and the mixture added in various proportions to the furfurin solution. An acetone-sulphuric acid mixture of 80 parts sulphuric acid to 100 parts acetone by volume produced excellent results when added to the furfurin solution in the ratio of 20 parts of acetone-acid mixture to 100 parts of furfurin solution.

A step farther in the use of acetone was to reflux the acetone and furfurin solution, and add acid to the refluxed mixture. The final formula developed for producing the resin is: (1) Reflux a mixture of 10 parts of a 20 per cent furfurin solution and 1 part acetone (by volume) for 20 minutes. (2) Cool to 30° C. and add slowly with stirring 1 part of concentrated sulphuric acid to 10 parts (by volume) of the refluxed mixture. Cool rapidly to 35 ° C., add fillers if desired, and place in molds. (3) Remove from molds as soon as solid enough to permit handling. Cracking of the material results if the article is permitted to remain in the mold for an extended period of time. The resin produced has a tensile strength in excess of 1,000 pounds per square inch. It is hard, glossy, does not crack, and is easily molded.

The refluxed acetone-furfurin solution plastic was used in the fabrication of small tanks. In this application of the resin 17 grams of shredded asbestos per 100 cubic centimeters of liquid material was used as a filler. A small size, the inside dimensions of which were 7½ x 4½ x 4½ inches, with half-inch walls, was first molded; later a larger tank, 18 x 6 x 6 inches, with a wall thickness of three-quarters of an inch, was produced. The outstanding feature of these tanks was their resistance to corrosion. Aqueous solutions of caustic, and sulphuric and hydrochloric acids did not corrode the material. Aqueous hydrofluoric acid was also kept in the tanks without damage by the acid. The resinous material did absorb some of the liquid; when the tanks were emptied and dried, the evaporation of the absorbed solution caused the walls of the tanks to warp and crack. This defect can be avoided by keeping the tanks filled at all times.

The addition of sulphur dioxide to furfural results in a solution which is polymerized to a soft, porous material by the addition of hydrochloric acid. The softness of the material enables it to be used as a crayon. The optimum amount of sulphur dioxide was found to be 8 grams per 100 grams of solution. The correct amount of hydrochloric acid is dependent upon the hardness desired; an increase in the amount of acid used decreases the hardness. The addition of furfural treated with oxy-
gen was found to be beneficial. The treatment with oxygen consisted of bubbling the gas through furfural heated to a temperature of 110° C. An excellent group of crayons may be produced by mixing 6 parts of an 8 per cent sulphur dioxide in furfural solution with 4 parts by volume of furfural treated with oxygen for 2 hours. This mixture is polymerized with amounts of concentrated hydrochloric acid varying from 10 to 100 per cent of the amount of furfural mixture used. By varying the acid used in 10 per cent intervals, a series of ten crayons having an excellent gradation in hardness is obtained. The crayons compare favorably with the charcoal pencils used for sketching purposes.

A plant for the production of the refluxed acetone-furfurin solution plastic has been designed. The plant is divided into two units, the first of which produces the furfurin solution, and refluxes it with the correct amount of acetone. The second unit is designed to mix continuously the sulphuric acid and refluxed acetone-furfurin solution in the desired ratio, and to cool the mixture rapidly to its molding temperature. Mixers are provided for the incorporation of fillers.
QUANTITATIVE STUDIES ON THE FORMATION OF XYLOSE FROM PENTOSAN-CONTAINING MATERIALS

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Accepted for publication August 1, 1934.

The utilization of agricultural products for the production of industrial chemicals is a subject of paramount interest in the field of chemical research at the present time. The pentosan-containing materials may be considered as by-products from the various agricultural industries. Some of these by-products which are produced in abundance include bagasse, cornstalks and cobs, cereal straws and hulls, peanut shells, cotton burs, and cottonseed-hulls. These materials, upon acid hydrolysis, yield up to 40 per cent reducing sugars, chiefly xylose. The purpose of this investigation was to make a study of the effect of acid concentration (HCl) and temperature (steam pressure) on the rate of hydrolysis and yield of xylose from oat-hulls, and to correlate the copper number with the production of solvents by the butyl-acetonic fermentation.

EXPERIMENTAL

ANALYTICAL METHODS

The reducing sugars formed by dilute acid hydrolysis of the pentosan-containing materials (oat-hulls) were determined by the method of Shaffer and Hartman.

APPARATUS

The apparatus consisted of a specially built autoclave (digester), constructed in such a manner that samples could be removed from the reaction medium for analysis without disturbing the temperature and pressure equilibrium. The essential parts of the digester were: (1) the reaction chamber which consisted of a five liter balloon flask; (2) motor driven mechanical stirrer; (3) small condenser connected to a sampling device; (4) a shell or autoclave that would operate under steam pressure up to 100 pounds per square inch.

SUMMARY OF RESULTS

The optimum conditions for hydrolysis of oat-hulls by dilute hydrochloric acid at various pressures are presented in the following table.

Studies were made on the liquid-solid ratio (cubic centimeters HCl solution per gram of oat-hulls) at 40 pounds steam pressure per square inch. It was shown that with increase in concentration of hulls there is a drop in yield of reducing sugars, and the time lag increases. That is, with increase in concentration of hulls the acid concentration must also be increased. For example, with a 5:1 ratio the optimum acid concen-

1Original thesis submitted June, 1934.
TABLE 1. Optimum conditions for hydrolysis of oat-hulls with hydrochloric acid at various pressures

<table>
<thead>
<tr>
<th>Liquid-hull Ratio</th>
<th>Pressure (Lbs./Sq. In.)</th>
<th>HCl normality</th>
<th>Time of heating in minutes</th>
<th>Reducing sugars percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 to 1</td>
<td>Atmospheric</td>
<td>2.000</td>
<td>180</td>
<td>39.50</td>
</tr>
<tr>
<td>10 to 1</td>
<td>20</td>
<td>0.100</td>
<td>120</td>
<td>40.00</td>
</tr>
<tr>
<td>10 to 1</td>
<td>40</td>
<td>0.050</td>
<td>90</td>
<td>40.00</td>
</tr>
<tr>
<td>10 to 1</td>
<td>60</td>
<td>0.042</td>
<td>75</td>
<td>39.50</td>
</tr>
<tr>
<td>10 to 1</td>
<td>80</td>
<td>0.042</td>
<td>60</td>
<td>39.90</td>
</tr>
<tr>
<td>10 to 1</td>
<td>100</td>
<td>0.042</td>
<td>30</td>
<td>40.50</td>
</tr>
<tr>
<td>5 to 1</td>
<td>40</td>
<td>0.070</td>
<td>150</td>
<td>36.00</td>
</tr>
</tbody>
</table>

A study was made of the carbon balance during hydrolysis of the oat-hulls. The data show that, during hydrolysis, about 45 per cent of the hull is dissolved with a yield of 39.1 per cent reducing sugars (xylose); the remaining unhydrolyzed residue amounted to 52.6 per cent. About 2.2 per cent of volatile products was lost during the hydrolysis. The hydrolysis was carried out under the following conditions: 20 pounds steam pressure per square inch, 10.1 ratio, 0.1383 N HCl, time of cook 90 minutes. The oat-hull and residue, after hydrolysis, were both analyzed for lignin, to determine its distribution. About 21.7 per cent of the lignin was dissolved or decomposed during hydrolysis. The distribution of ash was accounted for quantitatively. The residue after hydrolysis of the oat-hull was shown to consist mainly of lignin, crude fiber, and ash; these values total to 98.56 per cent. The oat-hulls were also analyzed for moisture loss at 105° C., ether solubles, and crude fiber. These data from the carbon balance studies indicate that xylose results from the hydrolysis of a C₅ compound rather than from a C₆ compound, and that volatile acids, particularly acetic, are formed in appreciable amounts.

Xylan was prepared from clean oat-hull meal, by extraction with 7 per cent sodium hydroxide solution and subsequent precipitation of the xylan by the addition of two volumes of 95 per cent alcohol. After filtration, the xylan was washed with alcohol and ether, dried in the air and stored in a tightly stoppered bottle. The xylan was analyzed for ash, moisture, lignin, and xylan by hydrolysis to xylose; the lignin was determined by difference. The xylan was hydrolyzed in the digester under similar conditions to those employed for the oat-hull hydrolysis. The results show that xylan is more readily hydrolyzed in the free state by dilute hydrochloric acid solution than when present in the hulls.

A correlation was made between the copper number and the production of solvents by the butyl-acetonic fermentation. Crude xylose solutions were prepared by hydrolyzing oat-hulls with 0.07 N HCl solution under 20 pounds pressure per square inch in the digester. A 5:1
ratio was used and a series of cooks were made (60, 90, and 150 minutes). The pH of these crude xylose solutions was regulated to 5.0 by the addition of saturated NaOH solution, and the quantity of reducing sugars (copper number) present, was determined. These solutions were then fermented and analyzed for total solvents according to the standard technique employed in this laboratory. The data indicate that the maximum yield of solvents is produced from solutions which have had less drastic treatment than is required for the maximum yield of reducing sugars. That is, the best results in fermentation will be obtained when the acid concentration, temperature, or time of hydrolysis are slightly less than those given for maximum yield of reducing sugars.
CONDENSATION REACTIONS OF FURFURAL AND ITS DERIVATIVES

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Accepted for publication August 1, 1934

PART I
POLYMERIZATION AND STABILITIES OF FURAN COMPOUNDS

Preliminary to investigations on actual condensation reactions, a series of studies was made on the stabilities of furfural and its derivatives.

By treating 1 cc. portions of furfural with one drop or a small fragment of 66 different inorganic reagents, it was found that the greatest decomposition and resinification was produced by the halides of elements which occur in the center of the Periodic Table. This decomposing activity was particularly noticeable with the halides of the Groups 3, 4 and 5.

In general, organic compounds were without marked action unless an active halogen or strongly acidic group were present. Amino compounds caused a slow decomposition. Furfuryl alcohol behaved in a manner similar to that of furfural.

Hydroquinone, pyrogallol and pyrocatechol previously recommended as stabilizers for furfural and certain furan compounds were found to be not only without stabilizing effect, but they actually accelerated decomposition of furfural when the furfural was sealed for three years with the so-called stabilizers. When the tubes of furfural with a little stabilizer were left open to the acidic atmosphere of the laboratory, pyrogallol, hydroquinone and pyrocatechol were found of value as stabilizers.

The best method for keeping furfural is to seal it in an inert atmosphere. It was found that furfural sealed with nitrogen kept for three years. It became brown in color.

Furfuryl alcohol sealed with air became only a golden-yellow in color after three years standing.

In a series of experiments, 34 different compounds were treated separately with each of the following reagents: phosphorus tribromide, silicon tetrachloride, phosphorus trichloride, sulfur monochloride and arsenic tribromide. Varying degrees of stabilities were noted. The influence of a miscellany of substituents in the furan ring was noted.

The interesting fact was noted that the more sensitive groups are, in general, those that contain a methylene group attached to the furan

1Original thesis submitted December, 1933. This work was directed by Dr. Henry Gilman.
3Other substances were also studied in regard to their stabilizing effect.
nucleus. The greatest stabilizing groups are nitro and carboxyl and groups derived from carboxyl.

COLOR REACTIONS

An attempt was made to obtain a satisfactory color reaction for the furan nucleus. A color reaction would give a means of readily determining the presence of the furan ring after a series of vigorous transformations. The tests with aniline acetate\textsuperscript{4}, vanillin\textsuperscript{5}, pine splints\textsuperscript{6}, isatin\textsuperscript{7} and ferric chloride\textsuperscript{8} have been found essentially unreliable and insufficient.

It was hoped that in the various treatments of furan compounds with various substances a satisfactory color reaction would result. No definite or uniform color was observed. Another attempt along similar lines has recently been reported\textsuperscript{9}.

PART II

The actual condensation reactions studied for furan compounds were the Friedel-Crafts and Gattermann-Koch reactions.

The first definite work done in the introduction of acyl groups by the Friedel-Crafts and Gattermann-Koch reactions was reported by Reichstein\textsuperscript{10} as recently as 1930. No attempt was made to alkylate furan compounds prior to a quite recent paper on the alkylation and acylation of furan compounds\textsuperscript{11}. This earlier paper\textsuperscript{11} reported the successful alkylation of alkyl furoates, furyl ketones and furfural. This latter substance gave an anomalous product that is being further investigated. It was found\textsuperscript{11} that alkyl furoates and furan could be acylated in good yields. Ethyl 2-methyl-5-furoate has been acylated\textsuperscript{12}.

DISCUSSION

Furan compounds, in general, show a greater ease of alkylation and acylation than than benzene or benzene compounds\textsuperscript{11}.

The 2-furyl alkyl ketones prepared as recently directed\textsuperscript{11} showed no hypnotic action. Those tested were furyl methyl, ethyl isopropyl, n-propyl, n-butyl, isobutyl and n-amyl ketones.

The 5-alkyl-2-furoic acids\textsuperscript{11} showed marked germicidal action\textsuperscript{13}. The acids studied were 5-methyl-, 5-isopropyl-, 5-tert.-butyl- and 5-amylfuroic acids.

Acetylation of 3,4-dicarbomethoxyfuran yielded 2-acetyl-3,4-dicarbomethoxyfuran.
All attempts to carry out a Friedel-Crafts substitution on a furan containing the nitro group were futile. In one case it was found possible to substitute a chlorine atom for the nitro group. That is, from nitro-furan, propionyl chloride and titanium tetrachloride in carbon disulfide there was isolated 2-chlorofuryl 5-ethyl ketone.

The general order of strength of various condensing agents in introducing alkyl groups into the furan nucleus by the Friedel-Crafts reaction is as follows:

\[
\text{AlCl}_3 > \text{FeCl}_3 > \text{SnCl}_4
\]

The relative arrangement with respect to acylation is the following:

\[
\text{SnCl}_4 > \text{FeCl}_3 > \text{AlCl}_3 > \text{TiCl}_4
\]

**EXPERIMENTAL**

The technique used throughout was that recently reported\(^1\). All alkylations, acylations by acid halides and acylations by acid anhydrides were carried out by the same general directions.

The 2-acetyl-3,4-dicarbomethoxyfuran from 3,4-dicarbomethoxyfuran with acetic anhydride and stannic chloride in benzene melted at 108°.

Methyl furoate was alkylated by butylene to yield methyl 5-tert-butyl-2-furoate.

The Gattermann-Koch reaction\(^1\) would not introduce a formyl group into 2-methyl-3-furoic acid or ethyl 2-methyl-3-furoate. This was predicted by the work of Reichstein\(^1\).

**SUMMARY**

1. The stability of a furan compound depends on the groups present.

2. The value of stabilizing agents depends on the conditions under which furanic substances are stored.

3. The Friedel-Crafts reaction will apply to certain furan compounds.

4. The Gattermann-Koch reaction did not introduce a formyl group into 2-methyl-3-furoic acid or ethyl 2-methyl-3-furoate.

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BACTERIOLOGICAL STUDIES ON BUTTER SHOWING SURFACE TAINT

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Surface taint is a butter defect that has been encountered so frequently it has been characterized as a definite abnormality and differentiated from other types of deterioration. It was first brought to the attention of the trade in Western Canada in 1919. What is essentially the same defect has been discussed in other countries under various names. Surface taint is a defect that develops after the butter is made and makes its first appearance at the surface of the butter and then gradually penetrates to the center. The odor and flavor of the defective butter suggest putrefaction. The taint ordinarily appears in the butter about ten days after manufacture when stored at a temperature of approximately 5° C.; at higher temperatures it may be evident in a few days.

The investigation showed that the numbers of bacteria, yeasts and molds in commercial surface taint butter were generally high and they were usually greater at the surface than in the interior of the butter. Many of the microorganisms were essentially the same as those from normal butter. In general, the types of bacteria found in surface taint butter were not unusual. Although the nature of the defect suggests proteolysis, a comparatively small percentage of these types of organisms were isolated by the methods used.

Acidity and lactose determinations of surface taint butter did not show any differences from those on normal butter. Soluble and amino nitrogen determinations of the surface and interior of the defective butter showed evidence of proteolysis. The results from steam distillations indicated that distillates secured from surface taint butter have a greater alkalinity than those from normal butter.

Surface taint butter inoculated directly into normal butter failed to reproduce the defect, but when inoculated into sweet cream, which had been previously pasteurized, and the cream churned, the resulting butter developed the taint in many instances when stored for three days at 15.5° C., or seven days at 5.5° C. By following this procedure the taint could often be reproduced down through a series of churnings.

The cultures to be studied in detail were selected on the basis of the production in milk of an odor that suggested proteolysis. The procedure of inoculating the pasteurized cream and churning it into butter proved to be the only effective means of reproducing the taint. An organism, tentatively designated *Achromobacter putrefaciens*, was isolated from several samples of surface taint butter. *A. putrefaciens* produced typical surface taint in experimental churnings of butter made from cream inoculated with it. This organism was apparently present in only small numbers in the butter from which it was isolated. Also, the organism

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1Original thesis submitted June, 1931. 

[145]
produced surface taint in butter when the numbers per milliliter were comparatively small. Organisms other than *A. putrefaciens*, which would produce surface taint, when inoculated into pasturized cream and the cream churned, were secured from a number of samples of surface taint butter. These organisms appeared to represent three other types and were tentatively designated types B, C and D. The study made indicates that type D belongs to the genus Achromobacter, while types B and C may possibly belong to the genus Pseudomonas.

Studies made with *A. putrefaciens* and one other type showed that their development, and consequently the development of surface taint, could be controlled by the amount of salt incorporated in the butter. Unsalted and low salted butter always developed the taint, while medium and high salted butter remained normal. Also, the use of butter culture was influential in restraining the development of surface taint. The butter culture was never permitted to ripen the cream, but 10 per cent was added just before churning. The heat resistance of *A. putrefaciens* and the other cultures studied showed that the ordinary temperatures of pasteurization were effective in killing the organisms.
STUDIES ON VITAMINS B AND G IN GROWTH AND LACTATION IN THE RAT

(a) THE EFFECTS OF EXTRACTS OF VITAMIN B AND G

(b) THE DISTRIBUTION OF VITAMIN G¹

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Experiments performed by Guest, Nelson, Parks, and Fulmer (1), Taylor (2) and investigators in other institutions have demonstrated that certain purified diets, which support normal growth in young rats, fail to supply enough of the water soluble vitamin B complex for lactation. Evans and Burr (3) and Sure (4) observed that three to five times as much yeast was required to supply the vitamin B complex for lactation as was necessary to produce normal growth in young rats. The experiments reported in this paper were designed to investigate the requirements of vitamins B and G during lactation and the distribution of vitamin G in some natural foods.

The basal ration used throughout these studies contained: casein 18, salts (185) 3.7, butterfat 4.0, cod-liver oil 1.0, and dextrin to 100 parts. If the material being studied was fed as a certain percentage of the ration, the dextrin content was reduced.

Pregnant females transferred from the stock ration to the experimental ration on the day of parturition were used in all lactation studies.

Several extracts and concentrates of vitamin B were made from dried yeast, wheat germ, and rice polishings by extraction with various mixtures of water and alcohol. Of the several vitamin B concentrates prepared an activated fuller's earth product, prepared as described below, was found to be the most satisfactory source of vitamin B to be used in lactation studies and in testing the vitamin G content of natural foods and vitamin G concentrates.

Ether extracted rice polishings were extracted with 95 per cent ethyl alcohol acidified with acetic acid. The extract was concentrated under reduced pressure. Lead acetate was added to give the maximum precipitate. This precipitate was filtered off and H₂SO₄ added to the filtrate to precipitate the lead. The pH of the lead free filtrate was adjusted to 4.5 and the vitamin B adsorbed on fuller's earth; so that one gram of fuller's earth was equivalent to 37.5 g. of rice polishings. Young rats grew at a normal rate with 0.053 g. of this activated fuller's earth as the source of vitamin B. Fifty-three thousandths of a gram of activated fuller's earth plus the basal ration produced an average gain of 2.5 g. per rat per week. In view of the findings of Evans and Lepkovsky (5) it is possible that the basal ration supplied some vitamin G; and the vitamin G content of the activated fuller's earth is less than is indicated by the gain of 2.5 g. per rat per week.

¹Original thesis submitted July, 1934.
Wilkinson and Nelson (6) observed that hog liver supplemented soybeans for lactation. Guha (7) found an aqueous ox-liver extract to be a potent source of vitamin G relatively deficient in vitamin B. Minced hog liver was extracted by the method used by Guha (7). The aqueous liver extract was concentrated in vacuo to 150 ml. per kilogram of liver. The effect of adding alcohol to this liver concentrate was studied. If ethyl alcohol was added to give a concentration of 50 per cent alcohol, little or no vitamin G was precipitated. As the concentration of alcohol was increased above 50 per cent the amount of vitamin G contained in the filtrate decreased, and the amount of vitamin G in the precipitate increased. The filtrates from precipitation with 80 or 90 per cent alcohol contained little or no vitamin G. The precipitates were potent sources of vitamin G, sufficiently free of vitamin B to produce polynierurities in rats.

Dried hog liver, aqueous extract of hog liver, or the precipitate formed by adding alcohol to a hog liver extract to give a concentration of 80 per cent alcohol were excellent sources of vitamin G. Whey powder and dried yeast were good sources of vitamin G. Wheat germ and rice polishings at a level of five per cent of the diet do not furnish sufficient vitamin G for normal growth. Wheat germ is a more potent source of vitamin G than rice polishings.

Wheat, oatmeal, yellow corn, white corn, and barley at a 60 per cent level, and rice polishings, rice bran, and wheat germ at a 10 per cent level are deficient in vitamin G for lactation. Ten per cent autoclaved yeast supplemented the various seeds and products from seeds to produce lactation at a superior rate. Dried hog liver at a level of 3.3 per cent supplements 50 per cent yellow corn as a source of vitamins B and G for lactation.

Wheat and yellow corn are supplemented by the precipitate formed by adding alcohol to a liver extract to give a concentration of 80 per cent alcohol. Activated fuller’s earth (source of vitamin B) does not supplement a wheat or corn ration for lactation. Females on rations in which 50 per cent yellow corn or 10 per cent rice polishings furnish the sole sources of vitamin B complex are unable to rear normal young. The feeding of a hog liver concentrate (source of vitamin G) to the young from the fourteenth to twenty-eighth day increased the weight of the young and decreased the mortality. These facts indicate that the females secreted milk and cared for their young, but the young failed to thrive, due to the low vitamin G content of the milk.

Dried hog liver, the precipitate formed by adding alcohol to an aqueous liver extract to give a concentration of 80 per cent alcohol, or autoclaved yeast are unable to support lactation as the sole sources of vitamins B and G. Activated fuller’s earth (source of vitamin B) was also unable to support lactation as the sole source of the vitamin B complex. In order to obtain lactation at approximately the normal rate, it was necessary to feed sources of vitamins B and G simultaneously and at about six times the rate required to produce normal growth in young rats. These data show that increased amounts of both vitamins B and G are required by the lactating female, in order to rear normal young.

In no case was lactation as successful when extracts and concentrates served as the sources of the vitamin B complex as when grains
plus autoclaved yeast, dried hog liver, or the precipitate formed by adding alcohol to a hog liver extract served as the sources of the vitamin B complex. Since these are the same sources of vitamin G as were used to supplement the fuller's earth, it is probable that the grains studied furnished some substance necessary for lactation in the rat that is not extracted with alcohol and adsorbed on fuller's earth.

LITERATURE CITED


SOME FACTORS AFFECTING THE GROWTH OF PENICILLIUM ROQUEFORTI IN CHEESE

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The manufacture of varieties of blue veined cheese, such as Roquefort and Wensleydale, originated as an art which has not been entirely successfully transplanted to other parts of the world. Therefore, a fuller knowledge of the factors which affect the growth of Penicillium roqueforti may put the manufacture of such cheese on a more scientific basis.

PART I

THE CITRATES OF MILK AND THEIR POSSIBLE FERMENTATION PRODUCTS AS THEY AFFECT THE GROWTH OF P. ROQUEFORTI

Studies were carried out on the effect of citric and acetic acids on the growth of strains of P. roqueforti isolated from Roquefort and Wensleydale cheese. In making these studies the cultures of the molds were inoculated into sweet skim milk and on plates of standard agar containing 0.272 and 0.1423 per cent of citric acid or 0.1313 and 0.048 per cent of acetic acid. The amount of growth in the milk media was determined by the amount of undigested casein present after 10 days incubation; while the diameter of a single colony on the plate, after comparative periods, was used as the index of growth on the agar. The incubation temperature ranged from 20° C. to 22.5° C.

Citric acid and acetic acid, in amounts comparable with those found in milk and starter, had an effect on the growth of different strains of P. roqueforti. In milk low concentrations of acetic acid tended to reduce the digestion of casein by strains of P. roqueforti, while citric acid tended to increase this digestion. On the other hand, in standard agar acetic acid increased the growth while citric acid tended to inhibit it. This work would indicate that the type of starter used in the manufacture of blue veined cheese might have a significant bearing on the subsequent growth of the mold in the cheese.

An investigation into the proportion of the citrates of milk incorporated in the curd during cheese making showed by analysis (using the method of Beau modified by Denige) of both milk and whey that there were not citrates in the cheese other than those associated with the whey incorporated. Citrates added to the milk for cheese making were found in the whey and not held by the curd. Therefore, the very small propor-
tion of citric acid or its decomposition products in the cheese is unlikely to be of significance in the ripening of cheese.

PART II

THE EFFECT OF AMMONIUM SALTS ON THE GROWTH OF P. ROQUEFORTI IN CHEESE

The work of Weisbrodt showed that NH₄Cl greatly increased the growth of P. roqueforti on standard synthetic media. Therefore, determinations of the ammonia in cheese were conducted according to a modification of Lisk's method. This method proved to be satisfactory since there was an almost complete recovery of the ammonia salts added to the cheese. Wensleydale cheeses of various ages were analyzed for ammonia. Very little ammonia was found in the fresh cheese, but a considerable quantity developed in the mature cheese.

Additions of NH₄Cl to the curd at salting, in the approximate proportions as found satisfactory by Weisbrodt in synthetic media, had a detrimental effect on the growth of P. roqueforti.

PART III

THE OXYGEN REQUIREMENT OF P. ROQUEFORTI IN CHEESE

The need of oxygen for the growth of P. roqueforti having been established by various investigators, experiments were conducted to determine what physical means of increasing the air supply in the Wensleydale could be used to hasten and increase the growth of P. roqueforti. A method of putting tubes into the cheese and sucking air into the cheese from the outside was attempted. The method did not prove to be satisfactory, owing to air leaking down on the outside of the tube when suction was applied. In general, good mold growth did not develop in the cheese to which suction had been applied, though in many cases mold had grown in the cheese around the tubes.

A method of forcing oxygen from an oxygen cylinder into the cheese, using the above method of putting tubes into the center of the Wensleydale cheese, was tried. Though some of the cheese developed mold, the method in general does not point to a satisfactory way of increasing the growth of P. roqueforti in cheese.

On the basis of Henry's law, on the relationship of pressure to the solubility of gases in water, a method of frequently changing the pressure of the air on a series of cheese in an enclosed cylinder was experimented with. Seven Wensleydale cheese which were submitted to an average reduced pressure of 616 mm. for 7 hours each day, twenty-three times in all, showed a slight mold growth in three cheese, while the control cheese showed no growth. A record of the CO₂ collected from the above cheese is given and shows that an average of about one gram of CO₂ was removed during each operation.

A second experiment with the same apparatus was conducted in which seven lots of cheese were divided into four groups:

A. Control.

B. Subjected to reduced pressure twice a week for six weeks.

C. Bandages removed and subjected to reduced pressure twice a week for six weeks.
D. Bandages removed and the cheese skewered from one end (28 holes, 1/16 of an inch) and then subjected to reduced pressure twice a week for six weeks.

Two significant results were obtained. The alternation of reduced and atmospheric pressure hastened the mold growth but did not permanently improve it.

Skewering the cheese produced a permanent increase in mold growth for the cheese in Group D, as shown by final scores.

A preliminary experiment to determine the possibility of reducing the CO₂ produced in the cheese by fermentation was conducted. The percentage of the sugar incorporated in the curd was lowered by adding acidified water to the milk. Interesting results affecting the process of manufacture of the cheese are given.

Although the cheese had not had time to develop mold when the results were recorded, it was noted that the control group was more acid in taste and firmer in texture than those made with diluted milk. The cheese made with diluted milk appeared to be of a type that would ripen to a more satisfactory Wensleydale cheese.
CAUSES OF SLOW ACID PRODUCTION IN BUTTER CULTURES

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From the Department of Dairy Industry, Iowa State College

Accepted for publication August 1, 1934

The object of the investigation was to determine the reason why some butter cultures, which appear to be normal on the basis of chemical, bacteriological and organoleptic tests, fail to produce acid at a normal rate when inoculated into freshly pasteurized milk. This abnormally slow acid development in butter cultures is apparently encountered wherever butter cultures are used. The results obtained in the investigation are presented in two parts.

Part I deals with the investigation of factors closely associated with the milk as a cause of slow acid development. The following factors were studied: (a) source of the milk, (b) organisms present in the milk, and (c) contamination from plant equipment.

The source of the milk had little effect on the time required for coagulation by butter cultures or *Streptococcus lactis* cultures, whether the milk was raw or pasteurized. This was the case with both herd milk and the milk from individual cows. The variations were so small that the source of the milk was considered unimportant as a cause of slow acid production in butter cultures. The greatest variations were those caused by pasteurizing the milk and by using different butter cultures and *S. lactis* cultures. Pasteurizing the milk at 82° C. for 30 minutes decreased the time required for coagulation about 30 minutes as compared with raw milk. The difference in coagulation time among various butter cultures and also among various *S. lactis* cultures was as much as six hours.

The organisms present in the samples of raw milk tested did not restrain the development of acid by a butter culture to any marked extent. Furthermore, six unidentified cultures isolated from the raw milk did not have any significant restraining action on the production of acid by a butter culture.

Contamination from plant equipment, with the samples of milk studied, was not important as a cause of variations in acid production by butter cultures. The variations in the samples were too small and too inconsistent to be of any significance. However, it was in connection with the study of contamination from plant equipment that large differences were first noted in the rates of acid production by apparently normal butter cultures. These differences, caused by exceptionally slow rates of acid production by large lot cultures as compared with mother cultures, led to a study of the butter cultures used as inoculating material.

Part II, dealing with the investigations of the butter cultures used as inoculating material, involved (a) examination of butter cultures, (b) examination of bacteria free filtrates from butter cultures, and (c) attempts to produce slow butter cultures experimentally.

*Original thesis submitted June, 1934.*
In the examination of butter cultures, it was found that the growth of some freshly inoculated butter cultures could be definitely restrained by the addition of small amounts of certain large lot cultures or mother cultures. This restraining effect was a more common characteristic of abnormally slow butter cultures (failing to show coagulation after 16 hours) than of normal cultures. Furthermore, a restraining effect was shown by a larger proportion of the large lot cultures studied than of the mother cultures. No contamination was found in the 23 slow acid producing cultures or in the 17 normal cultures studied (some of each group being inhibitory) by plating on tomato juice agar or by microscopic examination. The morphology of the cells appeared to be typical of butter culture organisms.

Bacteria free filtrates were obtained from butter cultures by filtering them through coarse filter paper, then passing the filtrates through grade N Berkfeld filters. Tests for sterility of a considerable number of the filtrates were made by inoculating one ml. into each of two tubes of sterile litmus milk and incubating one of the tubes at room temperature and one at 37° C., and also by making smears of the filtrates on two plates of beef infusion agar and two plates of tomato juice agar and incubating one of each of these at room temperature and one at 37° C. The litmus milk tubes did not show changes except in one case in which a slow acid development appeared. The contents of many of the tubes showing no changes were examined microscopically and no indications of growth were ever found. The agar plates did not show growth of any nature after four days incubation, except that on an occasional plate a mold colony developed.

When bacteria free filtrates obtained from certain slow and normal butter cultures were added to freshly inoculated portions of a butter culture or _S. lactis_ culture there was a definite restraining action on the development of acid and on the increase in the numbers of bacteria. Since 11 (58 per cent) of the 19 filtrates from the mother cultures and 22 (96 per cent) of the 23 filtrates from large lot cultures caused rather marked decreases in the percentages of acid produced by the test cultures, it appeared that large lot cultures were more likely to yield restraining filtrates than mother cultures.

When plates were poured with butter cultures or _S. lactis_ cultures containing bacteria free filtrates and colonies picked into tubes of litmus milk, the coagulation rates of the _S. lactis_ cultures appeared to be normal, and in the case of the butter cultures, there seemed to be a normal distribution of the organisms among the butter culture types. Carrying mixtures of a butter culture and each of a number of restraining filtrates through a series of seven transfers commonly restored the culture to a normal rate of growth, and attempts to increase the activity of filtrates by adding them to a butter culture and then recovering them when coagulation had occurred were unsuccessful. Heating to comparatively low temperatures for short periods seemed to destroy the restraining action of the filtrates.

Since it was found that large lot cultures, which are exposed to the air more than mother cultures in the process of making, yielded restrain-

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The term "bacteria free filtrate" is used to refer to a filtrate free from bacteria in the usual form.
ing filtrates more often than mother cultures, attempts were made to produce slow butter cultures experimentally by exposure to the air. The method consisted of exposing to the air the milk from which butter cultures were made, or of bubbling air through the milk, and then inoculating the milk with normal butter cultures. The growth of certain butter cultures inoculated into milk given such treatment was sometimes restrained, thus indicating a relationship between the air which was in contact with the milk before inoculation, and the abnormally slow growth of the butter cultures inoculated into the milk. The suggestion that the inhibitory principle may come from the air is in agreement with the idea that it is a form of living matter, since various forms of life are present in the air.
PLASTICS FROM HIGH PENTOSANOCYELLULOSIC MATERIAL

WILLIAM DONALD HARRIS

From the Department of Chemical Engineering, Iowa State College

Accepted for publication August 1, 1934

Since the development of a plastic molding compound from phenol and formaldehyde by Baekeland, the plastic field has grown rapidly. However, before plastics can be economically used to any great extent for large articles and in building construction the cost of manufacture must be greatly reduced. The work herein described has been directed toward the development of a cheap synthetic plastic from corncobs and oat hulls.

HISTORICAL

Several early workers (1, 2, 4) have observed that cellulosic materials will condense with phenol in the presence of an acid catalyst to give resinous products. Work on a corncob-phenol plastic was started at Iowa State College when Moscrip (3) and Williams (5) attempted to use the furfural content of corncobs to produce a resin with phenol, in the corn cobs and, thereby, obtain a plastic material. The plastic which they obtained gave considerable promise, and this work is a continuation of the research on plastics from these highly important cellulosic raw materials.

EXPERIMENTAL

The experimental work was divided into two sections. The first included the investigation of the nature of the reactions of the pure constituents of corncobs and oat hulls with phenol and cresol. Purified cellulose, pentosans, pentoses and lignins were caused to react with phenol under the influence of sulfuric acid, zinc chloride, sodium hydroxide and hydrochloric acid as catalysts. In the reactions of cellulose and phenol, the proportion of cellulose to phenol was varied from 2:1 to 2:3 with a ratio of sulfuric acid catalyst to phenol of 1:5. The temperature of the reactions was varied from 90° to 160° C. The lignin, pentosans and pentoses were treated in a similar manner.

The second part of the experimental work was devoted to the development of a standard process for producing a plastic material, and to methods for improving this material. The process is as follows:

1. Five parts by weight of ZnCl₂ or H₂SO₄ are dissolved in an equal weight of water and then combined with 100 parts of cresol or phenol. The mixture is then heated to boiling in an autoclave.

2. Ground corncobs, 30 parts, or the same weight of oat hulls are gradually introduced with stirring into the hot cresol mixture.

3. The autoclave is closed and the temperature kept at 140° C. for 3 hours.

1 Original thesis submitted June, 1934.
4. The pressure is then released and the reacted material is subjected to a vacuum distillation until the boiling point rises to 260-300° C., depending upon the desired melting point of the resin.

5. The resin is then run out into flat pans and cooled until solid.

6. The plastic molding compound is prepared by grinding together 40 parts of the powdered resin and 60 parts of a wood flour filler.

The effect of the stearic acid and zinc stearate on the plasticity of the molding material was studied by incorporating 2 per cent of either stearic acid or zinc stearate in the molding powder. The molding powder was also ground to pass a 200 mesh sieve to observe the effect of fineness on the plasticity.

The relation of molding temperature to strength of product was investigated by varying the molding temperature from 100° to 140° C. and testing the strength of the molded products on a Page impact testing machine.

A comparison was made of the effect, as fillers, of corncob flour, soybean meal, Sil-o-cel, calcium carbonate, fine and coarse asbestos, and wood flour on the water-resistance and strength of the molded products. Paraformaldehyde and hexamine were tried as hardening agents.

RESULTS

To sum up the results of the studies of the reactions of the cellulose, lignins, and pentosans with phenols, it was found that all would condense with the phenol in the presence of \( \text{H}_2\text{SO}_4 \), HCl or ZnCl\(_2\) as catalysts. The product in each case was a black, tarry material which could be made into a rather brittle, black resin by removal of volatile constituents. Cellulose was also found to act in another way; it would dissolve in the phenol and zinc chloride, the latter acting as a solvent instead of a catalyst.

In the development of the plastic molding compound, both zinc stearate and stearic acid improved the plastic properties slightly, but gave the molded products a greasy appearing surface. Fine grinding increased plasticity but decreased strength. The optimum molding temperature for a molding powder containing 40 per cent resin and 60 per cent wood flour was found to be approximately 140° C. Wood flour and asbestos were by far the best fillers on the basis of strength and water-resistance tests. Molded products containing asbestos filler, though not quite as strong as those containing wood flour, were much superior in water resistance.

Hexamine was found to increase the strength of a molded disc so that it withstood the impact of a 2 Kg. weight by dropping 26 cm., whereas, the untreated disc withstood a drop of only 11 cm. The addition of paraformaldehyde did not increase the strength.

CONCLUSIONS

1. Cellulose, pentosans, pentoses and lignins can all be condensed with phenol to give resinous products.

2. A plastic material can be made from the combined solvent actions of zinc chloride and cresol; however, it has the disadvantage of not being very water-resistant.
3. The advantage of using ZnCl₂, as catalyst, over H₂SO₄ is due to the lower corrosive action of the plastic in the molding operation.

4. Asbestos and wood flour were proven to be the best fillers of those tested.

5. The additional plasticity derived from the use of zinc stearate or stearic acid does not warrant their use.

6. The addition of hexamine was decidedly beneficial as it reduced sticking in the mold and increased the strength.

SUMMARY

The reactions of the pure constituents of corncobs and oathulls were studied in an attempt to understand their influence in the corncob-cresol or oathull-cresol plastics.

Cellulose, lignins and pentosans were all found to condense with phenol in the presence of an acid catalyst to give similar resinous products.

A standard procedure was developed for preparing a molding compound from corncobs and cresol.

The properties of the molding compound were improved by a study of the effect of the molding temperature; by experiments with the use of plasticizers; by an investigation of various fillers, and through experiments on the effect of paraformaldehyde and hexamine as hardening agents.

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4. NOVOTNY, E. E., AND C. J. ROMIEUX

5. WILLIAMS, G. T.
A STUDY OF CHLORINE STERILIZING COMPOUNDS

I. RELATIONSHIP BETWEEN pH AND OXIDATION POTENTIALS

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From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

The linear relationship between pH and oxidation potential has been shown to hold for Chloramine-T in all the concentrations used. The average slope was calculated to be $-7.425$. This linear relationship did not extend over the entire pH range. The limits through which the relationship was constant were determined by the concentration.

The oxidation potential, as displayed by active chlorine compounds, can only be considered as a measure of the intensity factor since the concentration of active chlorine appeared to have little effect upon the value of the potential developed. The oxidation potential was influenced by the presence of other salts in the menstrum.

The linear relationship for pH and oxidation potential can be extended to the inorganic calcium and sodium hypochlorates. The potential values for the inorganic hypochlorates were much larger than for Chloramine-T. A gradual change was noted in the oxidation potentials proceeding from 10 p.p.m. (active) to 500 p.p.m. (active). This change may be due to the large amount of inert material present. The inorganic hypochlorates having the general formula $(OCl)_m$ have been shown to have similar properties.

An explanation has been proposed, based on the electro-negativity of the $(R)$ group, to account for the wide difference in the nature of the organic and inorganic hypochlorates.

Due to the fact that the pH and oxidation potential were a linear function for the compounds used, equations have been developed by which the pH or oxidation potential can be calculated.

The results have shown that the oxidation potential and the time to kill 99 per cent of the bacterial spores were a linear function between pH 6.0 and 7.0. A consideration of oxidation potential as a guide to germicidal efficiency has developed certain limitations. A mechanism has been offered to explain the manner in which the cells were destroyed by active chlorine.

It has been shown that the time to kill 99 per cent of the bacterial spores was a linear function of the percentage of active chlorine present as the undissociated hypochlorous acid. This would tend to show that the active germicide was the undissociated molecule.

The rate of decolorization of mercurochrome has been shown to be a linear function of the oxidation potential, and also a linear function of the percentage of HOCl present as the undissociated molecule.

Mathematically, the bleaching of mercurochrome and the time to kill 99 per cent of the bacterial spores were both linear functions of the same unit, namely, the oxidation potential, and therefore were linear in respect to each other. Thus, the bleaching of mercurochrome afforded a

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1Original thesis submitted December, 1933.
means of developing a suitable test for the germicidal efficiency of active chlorine compounds.
THE DETERMINATION OF THE EFFECT OF MANGANESE AND SULFUR ON THE MALLEABILIZATION OF WHITE CAST IRON

ARNOLD P. HOELSCHER

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

This study was undertaken in order to obtain more data concerning the effect of manganese and sulfur on the rates of malleabilization or graphitization of white cast iron. Two basic compositions of white iron were chosen. The composition of one series was approximately 2.40 per cent carbon, 1.0 per cent silicon, 0.040 per cent sulfur, 0.15 per cent phosphorus, and manganese, varying from 0.006 per cent to 0.37 per cent. The composition of the second series was approximately: 2.10 per cent carbon, 1.12 per cent silicon, 0.040 per cent sulfur, 0.15 per cent phosphorus and manganese varying from 0.006 to 0.0397 per cent.

The malleabilization was carried out in two so-called “stages” of temperature. The first stage, or the high temperature, was 926° C. (177° F.) and the second stage, or low temperature, was 704° C. (1300° F.).

EXPERIMENTAL PROCEDURE AND DATA

The materials used in the preparation of these alloys consisted of Armco iron, graphite, ferro-silicon (46 per cent silicon), ferro-manganese (46 per cent manganese), ferro-phosphorus (25 per cent phosphorus), and ferrous sulfide (50 per cent sulfur).

About 5,000 grams of Armco iron were melted in a small Plumbago crucible by means of a 35 kv-a Ajax Northrup electric furnace. The graphite, ferro-silicon, ferro-phosphorus, and ferrous sulfide were then added in quantities to give the desired white iron compositions. Certain amounts of this were then remelted and ferro-manganese added to produce the desired percentage of manganese. The final bars poured were cast in individual sand moulds, in 12 inch lengths having five-eights inch diameter. A pouring temperature of 1316-1371° C. (2400-2500° F.) was used as determined by a Leeds and Northrup optical pyrometer. Before pouring, the sand moulds had been at room temperature for 48-50 hours and after pouring were broken after an interval of 30 minutes.

The analytical determinations were made by the following methods. The carbon was determined by direct combustion in oxygen with ascarite as the absorbent. The silicon method used was that of nitro-sulfuric acid dehydration. The manganese was determined by the sodium bismuthate method. The barium sulfate precipitation method was used for sulfur; and for phosphorus, the alkali-acid titration method.

Samples of about one to two inches in length of the various alloys were placed in a Hump annealing furnace which had previously been brought to a temperature of 926° C. (1700° F.). To avoid decarburization the samples were packed with graphite in small iron or carbon con-
tainers, but in spite of this precaution slight decarburization occurred at the surface.

Iron-constantan thermocouples were used and the temperature controlled and recorded by a Leeds and Northrup recorder. This temperature was frequently checked by a portable pyrometer indicator which in turn had been checked by a Leeds and Northrup Student Potentiometer (new model) and found satisfactory. The rate of malleabilization was determined by taking samples from the annealing furnace at definite intervals of time, with subsequent microscopic examination. The absence of all free cementite constituted the first stage.

Upon completion of the first stage of malleabilization at 926° C. (1700° F.), the samples were again placed in containers and packed with graphite. The containers with the samples were then placed in the furnace and heated to 926° C. (1700° F.), whereupon the power was turned off and the samples allowed to cool in the furnace in the second stage temperature, 704° C. (1300° F.). The rate of malleabilization of the second stage was again determined by withdrawing samples at definite time intervals. These samples were then examined microscopically for complete removal of pearlite.

In tables 1 and 2 the most representative values of this study are tabulated.

**TABLE 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Mn</th>
<th>S</th>
<th>Mn-2S</th>
<th>Mn/S</th>
<th>Hours at 925° C.</th>
<th>Hours at 704° C.</th>
<th>Total hours</th>
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<tbody>
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<td>0.038</td>
<td>-0.015</td>
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<td>80</td>
<td>89</td>
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<td>3.9-1</td>
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<td>31</td>
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<tr>
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<td>41</td>
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<td>10.2-1</td>
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**DISCUSSION OF RESULTS**

In plotting the total time of malleabilization as ordinate against the Mn, S ratio as abscissa, it was observed that the minimum for the 2.10 per cent carbon series was 4.6 and that the minimum for the 2.40 per cent carbon series was 5.4. In a comparison of the Mn-2S values to the total time of malleabilization, it was noted that for samples 44, 55, and 68, the
MALLEABILIZATION OF WHITE CAST IRON

TABLE 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Mn</th>
<th>S</th>
<th>Mn-2S</th>
<th>Mn/S</th>
<th>Hours at 926°C</th>
<th>Hours at 704°C</th>
<th>Total hours</th>
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<td>20</td>
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<td>1.6-1</td>
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<td>55</td>
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<td>0.042</td>
<td>-0.005</td>
<td>1.9-1</td>
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<td>60</td>
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<td>0.052</td>
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<td>41½</td>
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<td>7</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td>54</td>
<td>0.356</td>
<td>0.040</td>
<td>0.276</td>
<td>8.9-1</td>
<td>8</td>
<td>30</td>
<td>38</td>
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<tr>
<td>55</td>
<td>0.372</td>
<td>0.033</td>
<td>0.306</td>
<td>11.6-1</td>
<td>7</td>
<td>30</td>
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<tr>
<td>51</td>
<td>0.374</td>
<td>0.032</td>
<td>0.310</td>
<td>11.7-1</td>
<td>12</td>
<td>30</td>
<td>42</td>
</tr>
</tbody>
</table>

Mn-2S was 0.136 and the total time 33½ hours. These values were the average of the three. In the case of samples 70, 71, and 72, the average Mn-2S was 0.161 and the total time 28.8 hours. The values for the Mn, S ratios are in fair agreement with those of Yemenidjian (1) and those of Kikuta (2).

LITERATURE CITED

(1) **Yemenidjian**

(2) **Kikuta**
THE ROLE OF INORGANIC SUBSTANCES AND AMINO ACIDS IN THE REGENERATION OF HEMOGLOBIN IN THE RAT

HAVARD LAWRENCE KEIL

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

There has been considerable controversy as to whether other substances, both inorganic and organic, would replace copper as a hemotinic, since copper was found to act as a supplement to iron in the diet for the relief of nutritional anemia in rats (1). Investigators from several different laboratories have reported hemoglobin regeneration due to iron salts alone (2) in rats made anemic by an exclusive milk diet. Beard, Baker, and Myers (3) stated that the supplementation of a milk and iron ration with salts of manganese, arsenic, titanium, zinc, rubidium, chromium, selenium, mercury, vanadium, and cobalt also formed hemoglobin. Other workers observed a distinct hematopoietic action by feeding tryptophane, histidine, arginine, tyrosine, and glutamic acid along with iron to anemic animals (4) (5).

The purpose of this investigation was to find out whether or not hemoglobin could be synthesized in anemic rats, maintained upon a milk and iron diet, by the administration of various inorganic substances and amino acids. Other studies were made upon the daily copper and iron requirements of anemic rats and also the effect of insoluble copper and iron compounds upon nutritional anemia.

EXPERIMENTAL

The experimental animals were rendered anemic by feeding a milk diet. They were housed individually in galvanized iron wire cages and fed a basal diet of milk collected specially in glass in order to insure a food of low copper content. The inorganic materials investigated were tested for copper by means of spark spectograms. Precautions were taken in all experiments to eliminate copper contaminations which might have crept in due to faulty feeding and housing of the animals.

All rats were bled weekly from the tail and the percentage of hemoglobin determined by the Newcomer acid hematin method. Records of both growth and hemoglobin values were used to determine whether or not a substance was curative in nutritional anemia.

CONCLUSIONS

Pure iron salts, fed in low-copper milk, will not regenerate hemoglobin in anemic rats.

Inorganic compounds of titanium, manganese, vanadium, arsenic, germanium, zinc, chromium, tin, mercury, cobalt, silver, and gold do not act as hemotinics when fed along with iron as ferric chloride in low-copper milk.

1Original thesis submitted July, 1933.
Intraperitoneally injected inorganic compounds of nickel, zinc, germanium, manganese, vanadium, arsenic, titanium, selenium, mercury, rubidium, and chromium will not cause hemoglobin formation in anemic animals fed upon a low-copper milk and iron ration.

The addition of tyrosine, tryptophane, arginine, glutamic acid or aspartic acid to a low-copper milk and iron diet fails in hematopoiesis.

Anemic rats may be cured by intraperitoneal injections of 0.002 mg. of copper as copper sulfate along with 0.1 mg. of iron as colloidal ferric hydroxide administered daily.

Insoluble copper compounds such as cupric sulfide, cuprus oxide, and cuprous iodide are utilized by anemic animals for hemoglobin regeneration.

Intraperitoneal injections of iron alone, either as the chloride or citrate, will give a temporary relief to rats suffering with nutritional anemia.

Colloidal solutions of cupric hydroxide and ferric hydroxide administered simultaneously either orally or intraperitoneally will cure nutritional anemia in rats.

Iron in the form of ferric hydroxide suspension, when fed along with copper as copper sulfate, restores the hemoglobin level to normal.

Weak solutions of hydrochloric acid injected intraperitoneally into anemic rats give temporary relief.


THE BIOCHEMISTRY OF THE PRODUCTION OF 2,3-BUTYLENE GLYCOL BY FERMENTATION

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From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

One of the fields of research now occupying the attention of many agencies is the utilization of agricultural products, including the so-called farm wastes, as raw materials of the chemical industries. Among the many products formed by the action of micro-organisms upon the carbohydrates, the chemical 2,3-butylene glycol offers many possibilities as a useful industrial chemical. The production of this chemical by fermentation has been studied for several years in these laboratories. The object of this thesis was to extend these preliminary findings and to ascertain the conditions leading to the highest possible yield of 2,3-butylene glycol in a medium of known composition. The substrate employed was sucrose.

Most of the experimental work was done with the organism *Aerobacter pectinovorum*. After the optimum medium had been developed, the following organisms were tested: two strains of *A. cloacae*, two of *A. aerogenes* and one of *A. pectinovorum*. There was no significant difference in the yield of 2,3-butylene glycol produced by these different organisms of the genus *Aerobacter*.

The following factors were studied as to their effect upon the yield of 2,3-butylene glycol: pH; concentration of sucrose; concentration of magnesium sulfate; concentration of ammonium chloride; concentration of secondary potassium phosphate and concentration of calcium chloride. The general procedure was to vary one factor at a time, the others being held constant.

Each fermenting medium was adjusted daily to a definite pH by the addition of 1 M sodium carbonate solution under sterile conditions. The total acidity developed was calculated from the total amount of the carbonate added. The fermenting media were also analyzed for unfermented sugar and for the 2,3-butylene glycol produced. The sugar was determined by the Shaffer and Hartmann method. The method of analysis for the 2,3-butylene glycol was developed during the course of the research and consisted briefly in the following procedure:

To each flask, after fermentation was completed, as evidenced by the cessation of the formation of acid, was added 1½ cc. of 12 N sodium hydroxide. The alkali caused a precipitation of suspended material, including bacteria, leaving a clear solution for analysis. A 20 cc. portion of the clear supernatant liquid was placed, together with 21 grams of powdered potassium carbonate, in a glass extraction tube which was so constructed that it, together with a small funnel, could be suspended from an A. S. T. M. extraction apparatus, and hence was adapted for the continuous extraction of liquids with an immiscible solvent. Stirring the mixture until all the salt was dissolved gave approximately 26 cc. of a saturated potassium carbonate solution.

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1 Original thesis submitted June, 1934.
The temperature of the water bath was so regulated that about 2 drops of ether condensed each second (45-50° C.), and the extraction was continued for 5 days at this rate. This prolonged extraction was found advisable for complete removal of the glycol. The ether was then evaporated at 45-50° C. and the flask allowed to stand un-stoppered until attaining constant weight (about 15 hours). The amount of impurities in the glycol separated by this method is quite small, as was found when this fraction from a large amount of fermentation mixture was examined.

By the use of some carefully fractionated and remarkably pure glycol (B.P. 182.5° C. corrected), data were obtained on the readings of a dipping refractometer in various concentrations of the glycol in water, and it was found that the refractometer reading is a linear function of the concentration of the glycol. In order to check the gravimetric method, 20 cc. of water were added to the weighed residues from the extractions, and the solutions were analyzed by the refractometric method. In general, the refractometric method gives somewhat lower results than those obtained by weighing. In the data presented in this thesis, the yields of glycol represent the average of the values obtained by the two procedures.

The results of these experiments show that in an inorganic medium, for the maximum conversion of sucrose into 2,3-butylene glycol:

1. There is a definite optimum pH of about 6.2.
2. The most efficient conversion of the sugar occurs at a concentration of 8 per cent.
3. There is a definite optimum concentration of magnesium sulfate at 0.175 per cent.
4. Ammonium chloride is very essential and at least 0.3 per cent must be present. A concentration higher than 0.3 per cent does not give yields of glycol differing appreciably from the yield at 0.3 per cent.
5. There is a definite optimum concentration of secondary potassium phosphate at 0.175 per cent.
6. A trace of calcium chloride is essential, but any appreciable concentration is somewhat harmful; a 0.1 per cent concentration being slightly more harmful than a 1.0 per cent concentration.
7. Various species of the genus Aerobacter produce approximately the same yields of glycol under like conditions.
8. Under optimum conditions the yield of glycol amounts to about 50 per cent, by weight, of the sucrose fermented.
THE EFFECT OF CERTAIN BACTERIA ON THE RIPENING OF
CHEDDAR CHEESE MADE FROM PASTEURIZED MILK

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Accepted for publication August 1, 1934

In many parts of the United States the milk delivered to cheese factories is frequently of poor quality. Such milk may contain various types of microorganisms which not only cause undesirable flavors and textures in the cheese, but which are objectionable from the standpoint of public health. Pasteurization of milk of poor quality is a logical procedure to insure cheese of at least fair quality. Experience has shown, however, that cheddar cheese made from pasteurized milk rarely develops the full, characteristic flavor normally found in raw milk cheese of good quality. The pasteurized milk cheese, in addition to its usual lack of flavor, generally requires an extended ripening period to insure a normal degree of protein breakdown.

The destructive effect of pasteurization on certain of the bacteria necessary for bringing about normal cheese ripening probably accounts in part for the undesirable characteristics of pasteurized milk cheddar cheese. It is likely, therefore, that the addition of certain strains of bacteria to pasteurized milk used for cheesemaking would produce desirable chemical changes in the cheese with a corresponding improvement in flavor. The addition of special bacterial cultures in this manner also opens up many possibilities in connection with the development of new and desirable flavors in cheese of the cheddar type.

The effect of certain bacteria on the ripening of cheddar cheese made from pasteurized milk was studied with milk obtained from three dairies. The milk was inoculated with varying numbers of the test bacteria previous to the cheesemaking process, and the cheese was examined for the nitrogen distribution and the flavor at regular intervals during ripening. The bacteria used included several strains of Lactobacillus casei, and one strain each of Aerobacter oxytocum, Streptococcus liquefaciens, Streptococcus paracitrovorus, and an unidentified Micrococcus. Experiments were also carried out to determine the effect of adding 10 per cent raw milk to pasteurized milk used for making cheddar cheese. For making the cheese, 40-gallon steam-jacketed cheese vats were used, and longhorn cheese weighing approximately 12 pounds each were made.

Thirteen series of cheese were manufactured; each series contained three cheese manufactured at the same time from equal portions of a single lot of milk. Usually one cheese was made from raw milk, one from pasteurized milk, and one from pasteurized milk plus a milk culture of a test organism, or 10 per cent raw milk. In some cases, however, all three of the cheese in a series were made from pasteurized milk, and each of two portions of milk was inoculated with a milk culture of a test organism.

*Original thesis submitted June, 1934.
Cheese juice, for analytical purposes, was obtained directly from the cheese by submitting mixtures of finely divided cheese and sand to relatively high pressures. To extract the juice from cheddar cheese, 400 grams of cheese were first cut into thin shreds with a small soap grater. The shreds thus obtained were mixed by hand with 800 grams of fine sea sand. A hydraulic laboratory press with an iron cylinder attachment was used to press the juice from the cheese. For each extraction the hollow iron cylinder was entirely covered on the inside with a closely woven linen cloth, and the mixture of cheese and sand placed into the cylinder between felt filter pads. The cylinder was set on an iron plate at the base of the press, and as the pressure was slowly applied, the cheese liquid was forced out of the cylinder, through clearance spaces, on to a grooved outlet around the outer edge of the plate; from here it dropped into a beaker.

Changes in the nitrogen distribution in cheese were determined by chemical analyses of cheese juice after approximately one, five, ten and fifteen weeks of ripening at about 4° C. The chemical analyses included determinations of total nitrogen, amino nitrogen and various proteins and protein decomposition products soluble or insoluble in trichloracetic acid, ethyl alcohol, phosphotungstic acid and tungstic acid. The results obtained were expressed as the cubic centimeters of 0.1 normal acid equivalent to the nitrogen in 1 cc. of cheese juice. The cheese was scored for flavor at the same periods that chemical analyses were made.

A steady breaking down of the proteins during the ripening was shown by increases in all the nitrogen fractions determined. Cheese made from raw milk and cheese made from pasteurized milk inoculated with test bacteria or raw milk showed a more rapid and extensive breakdown than the control pasteurized milk cheese. Practically all of the control pasteurized milk cheese was characterized by having a flat flavor and a tough, rubbery body.

Four of the seven strains of L. casei, when added individually to pasteurized milk, appeared to improve the flavor and hasten the ripening of the resulting cheese. Two of the strains consistently produced a distinctly pleasing, buttery flavor which was very desirable. The addition of a strain of S. liquefaciens in small numbers to pasteurized milk produced well ripened cheese in a comparatively short ripening period, while the addition of relatively large numbers of these bacteria produced extremely bitter and soft-bodied cheese, the juice of which contained relatively large amounts of nitrogen soluble in trichloracetic acid and ethyl alcohol, but insoluble in phosphotungstic acid and tungstic acid.

When a culture of A. oxytocum was added to pasteurized milk, the resulting cheese had an unclean flavor and the juice contained relatively large amounts of nitrogen insoluble in trichloracetic acid. The addition of S. paracitrovorus or the Micrococcus appeared to slightly improve the flavor and hasten the protein breakdown of pasteurized milk cheese. The addition of 10 per cent raw milk to pasteurized milk produced cheese very similar to raw milk cheese in nitrogen distribution and flavor.

The juices of the cheese made from raw milk, the cheese made from pasteurized milk after the addition of desirable bacteria, were all characterized by the presence of large amounts and percentages of nitrogenous fractions which were soluble in trichloracetic acid but insoluble in ethyl
alcohol, and also by the presence of small amounts and percentages of nitrogenous fractions which were insoluble in trichloracetic acid, as compared to the juices of the control cheese made from pasteurized milk. It appears that the formation, during the ripening, of relatively large amounts of a compound or compounds soluble in trichloracetic acid and insoluble in ethyl alcohol, may be responsible in part for the characteristic flavor of high quality cheddar cheese.
PHYSIOLOGICAL AND TOXICOLOGICAL STUDIES ON INSECTS

Edward Rawson McGowan

From the Department of Zoology and Entomology, Iowa State College

Accepted for publication August 1, 1934

PART I. RESPIRATORY RESPONSES OF ADULT ORTHOPTERA TO CERTAIN GASES

A method of measuring the tracheal ventilation in insects is described and illustrated by a figure. The apparatus consists of two closed chambers, one enclosing the head and thorax of the insect and the other the abdomen. The insect is sealed in between the chambers so that air can only pass from one chamber to the other by being forced through the tracheal system of the insect. As the pressure is kept equal to atmospheric pressure at all times in both chambers, any air movement from one chamber into the other must have been produced by the breathing movements of the insect. The pressure and volume in each side of the apparatus is controlled by reservoirs in which the water level can be readily adjusted and horizontal capillary tubes which are closed by short columns of water. The water in the capillary tubes is free to travel in either direction along the capillary tubes and is so easily moved that it responds immediately to any movements of air produced by the insect in ventilating its tracheal system.

Adult female Chortophaga viridifasciata DeG., Arphia sulphurea Fab., Dissosteira carolina Linne, Melanoplus bivittatus Say, Melanoplus differentialis Thomas, and Hippiscus, species undetermined, were studied.

The respiratory movements of the insects produced a streaming movement of air through the tracheal system. The air was inhaled principally into the thorax and exhaled principally from the abdomen.

Adult female C. viridifasciata at 28° C. passed an average of 0.22 cc. of air through their tracheal system per minute per gram of body weight with a minimum of 0.12 cc. and a maximum of 0.33 cc.

Adult female C. viridifasciata exhaled an average of 20 per cent of the total CO₂ evolved from the thorax and 80 per cent from the abdomen at 23° C. If it can be assumed that all the air exhaled contains the same percentage of CO₂, it is evident that only part (about 80 per cent) of the air movement within the tracheal system of these insects is a through movement in the direction given above.

In 93 per cent of the tests 15 per cent CO₂ produced an increase in the rate of tracheal ventilation. The maximum increase for any period was slightly more than 2,000 per cent. Seventy-two per cent of the tests showed a reversal of the direction of air movement through the tracheal system.

One per cent CO₂ did not consistently increase the rate of tracheal ventilation nor reverse the direction of air movement through the tracheal system in a single instance.

Original thesis submitted December, 1933.
Sub-lethal concentrations of CS\textsubscript{2} and nicotine vapor usually increased the rate of tracheal ventilation.

High concentrations (0.2 per cent) of HCN produced a rapid fall in the rate of tracheal ventilation.

Concentrations of CS\textsubscript{2} and HCN which killed the insect slowly produced an increase in the rate of tracheal ventilation followed by a decrease as the gas rendered the insect less and less active.

The apparatus as operated in these tests recorded only the movement of air completely through the tracheal system. In many of the tests, especially with 15 per cent CO\textsubscript{2}, the insects inhaled and exhaled rapidly and deeply, but did not produce a correspondingly large movement of air into the thorax and out of the abdomen. This was most noticeable when the direction of air movement through the tracheal system was being reversed. First, the air movement in the normal direction would be reduced until it stopped entirely, but air was still rapidly inhaled and exhaled with each spiracle apparently performing equally as inhalatory and exhalatory orifices. After a short period of this type of breathing more air began to be exhaled from the thorax than was inhaled into the thorax, which was a reversal of the normal.

Nine references are cited.

PART II. TOXICITY OF PETROLEUM OIL MIXED WITH CERTAIN CHEMICAL COMPOUNDS TO LARVAE OF CARPOCAPSA POMONELLA LINNE

Young larvae of Carpocapsa pomonella Linne were placed on apples that had been sprayed with unemulsified petroleum white oil to which had been added some material to increase its toxicity to the larvae. The number of blemishes produced on the fruit by the larvae was then recorded.

The following materials were added to the white oil: tannic acid, nicotine, nicotine sulphate, goulac, 1-3-8 trinitronaphthalene, methyl salicylate, para-dibromobenzene, naphthalene, copper cyanide, copper oleate, ground pyrethrum, alpha-naphthylamine, iodine, beta-iodo-naphthalene, meta-dinitrobenzene, dibromo-ortho-cresol, para-chloroaniline, para-nitroiodobenzene, 3-5 dibromo-ortho-cresol, xylene, allyl-isothiocyanate, beta-chloronaphthalene, carbon tetrachloride, cetyl arsenite, piperine, toluene, ortho-toluidine, thymol, ortho-dichlorobenzene, fluorobenzene, barium stearate, aniline, tetra-chloroethylene, 2-4 dichloro-6-phenylphenol, alpha-nitroanipthalene, para-nitrochlorobenzene, 2-5 dichloroaniline, 1-2-4-5 tetrachlorobenzene, monochloronaphthalene, copper sulfocyanide, rotenone, arsenious oxide, ortho-nitrochlorobenzene, barium oleate, benzene, chloroform, 1-nitro-2-naphthol, lamp black and sodium arsenite, benzyl arsenic acid, thallous malonate, sodium cyanide, meta-dichlorobenzene, phenol, trinitro-resorcinol, ortho-phenyl-phenol, 9-10-dichloroanthracene, trihexachloronaphthalene, menthol, para-dichlorobenzene, sodium sulfocyanide, dinitro-phenol, sodium-ortho-phenylphenyl, phenyl-iso-thio-cyanate, triphenyl-arcsine, beta-bromonaphthalene, dichloronitrobenzene, para-para-dichlorodiphenol, ortho-cresol, beta-naphthol, 2-chloro-6-phenyl-phenol, 3-5-dinitro-ortho-cresol, chloropicrin, bromopicrin, bromine, chloride, nitrobenzene, pyridine, sodium-chloro-ortho-cyclohexyl-phenate, para-dimethylaminobenzylaldehyde, cyanamide, bromoform, 1-2-4-trichlorobenzene, dinitronaphthalene, phenyl-
PHYSIOLOGICAL AND TOXICOLOGICAL STUDIES ON INSECTS

alpha-naphthylamine, ortho-cyclo-hexylphenol, phenacyl chloride, di-phenylaminooarsine, ground red pepper, anthracene, cumidine, chloroacetone, 1-3-5 trinitrobenzene, 2-4-dichlorophenol, beta-chloroethyl-paratoluene sulfonate, bromo-beta-naphthol, cetyl fluoride, carbon disulfide, iodoform, sodium fluoride, 2-4 dinitrochlorobenzene, cyanogen bromide, 2-4-dinitrobromobenzene, cetyl alcohol, metallic arsenic, para-bromobenzonitrile, beta-naphthylamine, para-nitro-bromobenzene and para-toluidine. These materials were tested in various mixtures in the white oil. The compounds are listed roughly in the order of their toxicity to the larvae. The concentration of the material in the oil varied from 1 per cent to 25 per cent.

Of the mixtures tested, nicotine, nicotine sulphate, 1-3-8 tri-nitronaphthalene, methyl salicylate, copper cyanide, copper oleate, alpha-naphthylamine and iodine were the most toxic at concentrations of 2 per cent or less in white oil. Of these materials, nicotine and nicotine sulphate mixtures were the only ones that gave more than 71 per cent control.

Thirty-six series of compounds were studied. Plant extract mixtures were the most toxic. Nicotine was the most toxic material tested in this series.

The copper mixtures tested possessed marked toxicity to the larvae. Copper cyanide was the most toxic material.

Barium, nitro-naphthalene and hydroxyl bromine mixtures and beta-iodo-naphthalene ranked next in toxicity. Barium stearate was the most toxic barium compound tested. 3-5 di-bromo-ortho-cresol was the most toxic at low concentrations of the bromine compounds.

Iodine, chlorine and bromine and some of their compounds ranked in the order listed in toxicity to codling moth larvae in this series of tests. Derivatives of naphthalene and naphthalene mixtures were slightly more toxic than derivatives of benzene and benzene mixtures in general. Anthracene and 9-10 dichloroanthracene were practically non-toxic to codling moth larvae.

Aromatic compounds were more toxic than aliphatic compounds. Cetyl arsenite was more toxic than the arsines or inorganic arsenic mixtures that were tested.

Thirty-five references are cited.
A STUDY OF THE CORYNEBACTERIA (DIPHTHEROIDS) ASSOCIATED WITH DISEASES OF DOMESTIC ANIMALS

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During recent years many contributions have been made concerning the relationship of diphtheroid bacteria to animal diseases, but until the time of this study the various species had not been correlated satisfactorily. In order to secure an adequate relationship of the different species of Corynebacteria associated with animal diseases, the literature was completely reviewed and 49 strains of Corynebacteria were examined. The study of these 49 cultures was restricted to morphology, staining reactions, cultural characteristics, biologic reactions, fermentation and agglutination. On the basis of the characteristics of the cultures, 9 were found to be Corynebacterium pyogenes, 12 were Corynebacterium pseudotuberculosis, 20 were Corynebacterium renalis and one was Corynebacterium equi. Two cultures isolated from milk were not included with any of the above four species, and two non-chromogenic, rapidly-growing strains also were placed in a separate type. Three cultures which appeared to be variants were considered to be different than the others, although they originated from Corynebacterium pseudotuberculosis and Corynebacterium renalis.

Photographs showing the morphology of the four species mentioned above and also their colony characteristics are to be found in the original thesis.

The most distinctive characteristics of Corynebacterium pyogenes were found to be: morphology; aerobic as well as anaerobic growth; reproduction only in media containing blood, blood serum or milk; coagulation of milk with subsequent digestion of the curd; liquefaction of gelatin and coagulated blood serum; production of acid in dextrose, levulose, galactose, mannose, sucrose, lactose, maltose and dextrin and by some strains, xylose.

Corynebacterium pseudotuberculosis was characterized by its dry granular colonies; pellicle formation on fluid media; inability to alter milk, gelatin and coagulated blood serum. It produced acid in dextrose, levulose, mannose, sucrose and maltose.

It was found that Corynebacterium renalis grew in moist isolated colonies, digested milk casein producing an alkaline reaction, but had no action on gelatin or solidified blood serum. It fermented dextrose and some strains also fermented levulose and mannose.

Corynebacterium equi was recognized by its ability to produce a red-colored pigment, its profuse viscid growth, reduction of nitrates and inability to ferment carbohydrates.

1Original thesis submitted July, 1933.
None of the four species demonstrated a close serologic relationship except _Corynebacterium pseudotuberculosis_ and _Corynebacterium renalis_. These two species showed marked cross agglutination.

Orange colored variants were isolated from three cultures. These variants resembled their parent cultures in morphology but were smooth and pigmented and failed to ferment carbohydrates, whereas the parent cultures were usually rough, cream colored and demonstrated some fermenting ability.

**CLASSIFICATION**

It was found that all four of the species had been named correctly by previous investigators. _Corynebacterium pyogenes_ was first called _Bacillus pyogenes_ by Glage, but Eberson gave it its present name in 1918. The correct designation for the organism was considered to be _Corynebacterium pyogenes_ (Glage) Eberson.

_Corynebacterium pseudotuberculosis_ was first named, in 1894, by Preisz, _Bacillus pseudotuberculosis ovis_. In 1918, Eberson gave it the name _Corynebacterium pseudotuberculosis_. It was believed that Bergey et al used an incorrect name in 1923, 1925 and 1930 when they proposed _Corynebacterium ovis_.

The name, _Corynebacterium renalis_, has not appeared in much of the recent literature even though Jones and Little, in 1926, described the organism quite adequately without making reference to its name. Enderlen described the organism in 1891, giving it the name _Bacillus renalis bovis_. The same year Höflich also described it and proposed the name _Bacillus pyelonephritidis boum_. Bollinger, however, made a report in 1890 upon the work of Enderlen and suggested the name _Bacillus renalis bovis_. Ernst gave the organism the name _Corynebacterium renalis_ in 1906. Since that time, Ford, in 1927, has been the only writer to consider the organism a distinct species.

Magnusson named _Corynebacterium equi_ in 1923. Lutje named it _Corynebacterium pyogenes (equi) roseum_ in 1924. He evidently was not aware of the previous description given by Magnusson. The organism has received rather complete description in the United States by Dimock and Edwards.

The type of variation encountered in the study was of great interest, because of the possible relationship of the variants to the organisms isolated by Daines and Austin from the skin lesions of tuberculin reacting cattle.
I. SOME FACTORS AFFECTING THE PRODUCTION OF INSULATION BOARD

II. THE DEVELOPMENT OF THE COMMERCIAL PRODUCTION OF REFRIGERATION BOARD AND PRESSBOARD

Theodore R. Naftziger

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Accepted for publication August 1, 1934

The problem was undertaken to further the production of synthetic lumber from cornstalks. The problem was divided into: first, factors affecting the production of insulation board, and second, the development of refrigeration board and pressboard.

EXPERIMENTAL

PART I

The first step of the investigation was to analyze the commercial boards for rosin, and the percentage of ash. The percentage of rosin was determined in each board. The percentage of ash was secured and the ash was analyzed for $\text{SiO}_2$, $\text{Fe}_2\text{O}_3$, and $\text{Al}_2\text{O}_3$. The boards were then subjected to various humidity conditions in order to study their respective expansion and contraction. Samples of each board were measured both before and after being exposed to the following: various humidities; drying to constant weight; immersion in water; and change in temperature at various humidities. Sodium silicate, ammonium chloride, ammonium acid phosphate, ammonium fluoride, ferrous sulphate, and various potassium salts were each added to cornstalk pulp as a possible fireproofing agent. A commercial fireproofing salt was also used in this work. Samples of commercial wall board were subjected to conditions suitable for mold growth. Various chemicals were added to the cornstalk pulp in order to inhibit mold growth.

EXPERIMENTAL

PART II

(A)

Various refining methods were used to produce a suitable refrigeration board from cornstalks. Boards were made from the various physical constituents of the corn plant. A suitable method for extracting the pith from the cornstalk was developed. A beater equipped with a revolving screened cylinder removed the pith from the beater. The pith was refined lightly in a jordan and then formed into boards and dried without pressure. The pithboard required a long time to dry since large amounts of water were retained. Such drying methods as: air drying; steam oven drying; vacuum drying; forced air drying; and electric drying were in-
vestigated. An apparatus was built in order to test the insulating value of pithboard.

(B)

The production of a hard, dense pressboard was undertaken. Such relationships as: beating time to strength; fiber length to strength; rod-mill and claflin refining to strength; and freeness to strength were determined. Different methods of drying the wet mat before pressing were employed. The effect of various pressures; length of time of pressing; and temperature of pressing, on strength, were secured. Various sizing materials were used for sizing pressboard. Each of the following sizing materials; rosin and alum; paraffin and alum; sodium alginate; paraffin emulsions; and asphalt emulsions, were investigated. Parawax, paraffin oil, Halowax and Bakelite varnish were used as possible surface sizes.

CONCLUSIONS

PART I

NuWood and Insulite contained more rosin than any of the other commercial boards, while Celotex and cooked Maizewood contained the least. Insulite seemed to be waterproofed better than the rest of the boards. NuWood was the poorest sized board. Masonite yielded the lowest ash, while Maizewood yielded the highest. Boards made from wood showed less ash than boards made from other fibrous materials. The commercial boards, which yielded high ash, also yielded high silica. The ash from cooked Maizewood proved to be 75.8 per cent silica. The boards made from materials other than wood seemed to contain less aluminum oxide than those made from wood. Insulite was twice as strong as the other boards. The majority of the boards tested around three hundred and fifty pounds to the square inch. Masonite seemed to expand more, both in length and in width, while Maizewood expanded the least when placed at laboratory conditions. Celotex showed the least amount of expansion in length and width both at the one hundred and the seventy per cent humidity. Little difference was noticed in the expansion of the unsanded Maizewood and the sanded Maizewood boards. No appreciable change in length or width was noticed on finished boards during the first hour after they were removed from a constant temperature oven. The boards attained their original length and width after standing twenty-four hours at a temperature of 80° F. and thirty per cent humidity. Maizewood lath expanded 0.78 per cent in length and 0.69 per cent in width when immersed in water for twenty-four hours. The lath contracted 0.38 per cent in length and 1.00 per cent in width after drying five days at 80° F. and thirty per cent humidity. Boards containing from four to six per cent moisture seemed to expand and contract less when removed from the dryer than did boards containing a higher or lower percentage of moisture. Boards containing high percentages of newsprint expanded the same amount as boards containing less newsprint. Temperature seemed to play a very little part in expansion at each humidity. Maizewood expanded more in length than any other commercial board. No difference was noted in width. Boards could not be fireproofed by adding different amounts of sodium silicate to the pulp in the beater.
Boards could be fireproofed by applying ammonium phosphate and other chemicals to the surface. Maizewood was fireproofed one hundred per cent by adding a commercial fireproofing compound to the pulp in the mixing tanks. The boards were not waterproofed sufficiently after the treatment was completed. Commercial wall boards molded if they were placed in a warm, moist atmosphere, but not before fifty to sixty days. The edges showed signs of molding before the surfaces. Boards treated with zinc chloride, copper sulphate, and mercuric chloride did not mold for some weeks.

PART II

The whole stalk furnished a fair grade of refrigeration board, but not nearly as good as the pith alone. A very good grade of pith could be separated from the outer fiber by the flotation method. Eighty per cent of the pith could be removed without much cortex adhering. Refrigeration board could not be dried in air commercially, due to the long time required for drying. Boards dried in the steam oven were of good quality. This method of drying was also expensive, for the efficiency of the dryer was only 24.9 per cent. Vacuum drying increased the efficiency somewhat, but it still was too expensive a process. Forced air drying seemed to give the best quality boards even though they did dry a little slower than any of the rest. Electricity proved to be very expensive for drying pithboard.

Cooking improved both the appearance and strength of the pressboard. Much time was saved by refining the pulp in a rodmill and claflin. The strongest boards were produced from pulp containing 66 per cent or more moisture. A wet mat still contained 54.5 per cent moisture even though it was pressed at eight hundred and seventy-four pounds per square inch. Thirty minutes was a sufficient length of time for pressing boards of one-eighth inch thickness. The strength of boards pressed at four hundred pounds per square inch was very little different from the strength of those boards pressed at one hundred and forty pounds. A high percentage of size weakened the strength of the pressboard. Boards were waterproofed by means of paraffin emulsions. Sodium alginate proved to be a very poor sizing material. Asphalt and pitch did not waterproof the board as well as paraffin. The strength was decreased in every case where any of three sizes were used. Surface sizing proved to be almost worthless. A Bakelite varnish coated board presented a very pleasing appearance.
I. FILTERABILITY OF HOG CHOLERA AS AFFECTED BY THE HYDROGEN ION CONCENTRATION

The filters selected for these studies included the standard laboratory filters such as the regular Mandler, Berkfeld No. 3 "N", Chamberland-Pasteur "F" and the Seitz. Gypsum filters made according to Kramer's formula and compound filters made of a gypsum cortex built around Berkfeld No. 3 "W" filter candles were also used in these studies. The pH determinations were made with the glass electrode. The experimental results are summarized in the following tables.

CONCLUSIONS

1. Serum virus of hog cholera passes readily through the regular Mandler, Berkfeld "N", Chamberland-Pasteur "F" and Seitz filters, which are all standard. It also passes readily through the gypsum and compound filters which were made in the laboratory.

2. These filters were efficient in removing bacteria from liquid suspensions.

3. The volume of the filtrate delivered by individual filters of the same type under identical pressures varied considerably.

4. The studies on the effect of pH upon the filterability of hog cholera serum virus showed that within the range used, pH = 5.0 to 9.0; this factor is not of practical significance.

II. THE MIGRATION OF HOG CHOLERA VIRUS WHEN SUBJECTED TO ELECTROPHORESIS

The apparatus used was similar to that used by Todd, with some modifications. The two side arms of a large three-way stopcock were turned to a vertical position two inches from the center tap. Pyrex test tubes, 2 x 15 cm., were drawn out from the base and welded to the three arms of the stopcock in a vertical position. The side chambers of the apparatus were connected by two inverted U tubes, with two small glass
<table>
<thead>
<tr>
<th>Filter</th>
<th>Serum virus</th>
<th>pH</th>
<th>Volume delivered</th>
<th>Temperature</th>
<th>Test pig</th>
<th>Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mandler (regular)</td>
<td>3903</td>
<td>5.0</td>
<td>45 cc.</td>
<td>22° C.</td>
<td>3928</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Mandler (regular)</td>
<td>3903</td>
<td>5.0</td>
<td>45 cc.</td>
<td>22° C.</td>
<td>3929</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Mandler (regular)</td>
<td>3903</td>
<td>5.0</td>
<td>45 cc.</td>
<td>22° C.</td>
<td>3930</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Mandler (regular)</td>
<td>3901</td>
<td>9.0</td>
<td>45 cc.</td>
<td>24° C.</td>
<td>3901</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Berkfeld &quot;N&quot;</td>
<td>3931</td>
<td>5.0</td>
<td>67.5 cc.</td>
<td>24° C.</td>
<td>3904</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Berkfeld &quot;N&quot;</td>
<td>3930</td>
<td>6.0</td>
<td>124.8 cc.</td>
<td>21° C.</td>
<td>3934</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Berkfeld &quot;N&quot;</td>
<td>3934</td>
<td>7.0</td>
<td>102.2 cc.</td>
<td>24° C.</td>
<td>3949</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Berkfeld &quot;N&quot;</td>
<td>3942</td>
<td>8.0</td>
<td>86.4 cc.</td>
<td>29° C.</td>
<td>3905</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Berkfeld &quot;N&quot;</td>
<td>3942</td>
<td>9.0</td>
<td>101.6 cc.</td>
<td>29° C.</td>
<td>3951</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Chamberland-Pasteur &quot;Fm&quot;</td>
<td>3942</td>
<td>5.0</td>
<td>43.5 cc.</td>
<td>31° C.</td>
<td>3950</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Chamberland-Pasteur &quot;Fm&quot;</td>
<td>3909</td>
<td>5.0</td>
<td>51.2 cc.</td>
<td>23° C.</td>
<td>3957</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Chamberland-Pasteur &quot;Fm&quot;</td>
<td>3909</td>
<td>6.0</td>
<td>51.2 cc.</td>
<td>23° C.</td>
<td>3958</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Chamberland-Pasteur &quot;Fm&quot;</td>
<td>3971</td>
<td>8.0</td>
<td>42.0 cc.</td>
<td>23° C.</td>
<td>3972</td>
<td>8th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Chamberland-Pasteur &quot;Fm&quot;</td>
<td>3971</td>
<td>9.0</td>
<td>54.1 cc.</td>
<td>23° C.</td>
<td>3979</td>
<td>8th day</td>
<td>H. C.</td>
</tr>
</tbody>
</table>

Vacuum maintained at 350 mm. Hg.
Time—3 minutes.
H. C. = positive diagnosis of hog cholera.
### TABLE 2. Experiments with gypsum filters

<table>
<thead>
<tr>
<th>Filter</th>
<th>Serum virus</th>
<th>pH</th>
<th>Volume delivered</th>
<th>Temperature</th>
<th>Test pig</th>
<th>Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gypsum</td>
<td>3971</td>
<td>5.0</td>
<td>14.1 cc.</td>
<td>23° C.</td>
<td>3978</td>
<td>9th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Gypsum</td>
<td>3979</td>
<td>6.0</td>
<td>16.5 cc.</td>
<td>22° C.</td>
<td>3967</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Gypsum</td>
<td>3971</td>
<td>7.0</td>
<td>12.2 cc.</td>
<td>23° C.</td>
<td>3976</td>
<td>7th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Gypsum</td>
<td>3971</td>
<td>8.0</td>
<td>18.2 cc.</td>
<td>22° C.</td>
<td>3977</td>
<td>12th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Gypsum</td>
<td>3977</td>
<td>9.0</td>
<td>15.4 cc.</td>
<td>20° C.</td>
<td>3996</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
</tbody>
</table>

Vacuum maintained at 350 mm. Hg.
Time—20 minutes.
H. C. = Positive diagnosis of hog cholera.
<table>
<thead>
<tr>
<th>Filter</th>
<th>Serum virus</th>
<th>pH</th>
<th>Volume delivered</th>
<th>Temperature</th>
<th>Test pig</th>
<th>Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>3996</td>
<td>5.0</td>
<td>36.5 cc.</td>
<td>21° C.</td>
<td>3998</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Compound</td>
<td>3996</td>
<td>6.0</td>
<td>41.3 cc.</td>
<td>21° C.</td>
<td>3999</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Compound</td>
<td>3999</td>
<td>7.0</td>
<td>34.8 cc.</td>
<td>22° C.</td>
<td>4000</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Compound</td>
<td>4708</td>
<td>8.0</td>
<td>44.2 cc.</td>
<td>20° C.</td>
<td>4003</td>
<td>10th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Compound</td>
<td>4708</td>
<td>9.0</td>
<td>40.1 cc.</td>
<td>22° C.</td>
<td>4010</td>
<td>4th day</td>
<td>H. C.</td>
</tr>
</tbody>
</table>

Vacuum maintained at 350 mm. Hg.
Time—20 minutes.
H. C. = Positive diagnosis of hog cholera.
TABLE 4. Experiments with Seitz-Uhlenhuth type of filters

<table>
<thead>
<tr>
<th>Filter</th>
<th>Serum virus</th>
<th>pH</th>
<th>Volume delivered</th>
<th>Temperature</th>
<th>Test pig</th>
<th>Symptoms</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seitz-Uhlenhuth</td>
<td>4010</td>
<td>5.0</td>
<td>14.0 cc.</td>
<td>21° C.</td>
<td>4711</td>
<td>7th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Seitz-Uhlenhuth</td>
<td>4714</td>
<td>6.0</td>
<td>16.4 cc.</td>
<td>22° C.</td>
<td>4715</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Seitz-Uhlenhuth</td>
<td>4714</td>
<td>7.0</td>
<td>18.6 cc.</td>
<td>20° C.</td>
<td>4716</td>
<td>5th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Seitz-Uhlenhuth</td>
<td>4716</td>
<td>8.0</td>
<td>15.2 cc.</td>
<td>21° C.</td>
<td>4726</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
<tr>
<td>Seitz-Uhlenhuth</td>
<td>4716</td>
<td>9.0</td>
<td>16.5 cc.</td>
<td>21° C.</td>
<td>4727</td>
<td>6th day</td>
<td>H. C.</td>
</tr>
</tbody>
</table>

Vacuum maintained at 350 mm. Hg.
Time—5 minutes.
H. C. = Positive diagnosis of hog cholera.
bottles containing non-polarizable electrodes. These tubes were filled with 1 per cent saline in 2 per cent agar. The cathode made of copper wire was submerged in dilute copper sulphate solution. The anode constructed of iron wire was submerged in a solution of ferric sulphate. The apparatus was sterilized after the cotton plugs had been placed in the lower side arm tubes. The sterile buffer solutions adjusted to the desired pH were placed in the side chambers of the apparatus. The serum virus after being adjusted to the proper pH was placed in the center chamber. The direct current was supplied by a motor generator and the current was measured by a milliammeter. The pH values were determined by a glass electrode. The experimental results are summarized as follows:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pH</th>
<th>Positive pole</th>
<th>Center chamber</th>
<th>Negative pole</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.0</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>6.0</td>
<td>+</td>
<td>*–</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>7.0</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>IV</td>
<td>8.0</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>V</td>
<td>9.0</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

+ Indicates the presence of active hog cholera virus as determined by inoculation into susceptible pigs.

— Indicates the absence of active hog cholera virus.

* Experimental pig immune to hog cholera.

Milliamps—20.

Time—3 hours.

Temperature—37° C.

The protein tests showed the presence of serum proteins in the liquid taken from the center chamber of the apparatus and also in that which was taken from the positive pole.

CONCLUSIONS

1. The serum virus of hog cholera migrates toward the positive pole at pH values from 5.0 to 9.0.
2. The virus of hog cholera either carries a negative electric charge or is carried toward the positive pole by the associated proteins.
3. It is not possible within the pH range studied to separate hog cholera virus from the associated proteins by the electrophoretic method employed.

PART III. EXPERIMENTS ON THE ATTENUATION OF VIRUS AND THE PRODUCTION OF IMMUNITY TO HOG CHOLERA DESICCATED TISSUES

Brain and spinal cord tissues were taken from a cholera infected pig and desiccated. When this preparation no longer proved virulent a sus-
ceptible pig was injected with two 10 cc. units of a 50 per cent suspension in a diluting agent consisting of equal parts of glycerine and physiologic saline solution. These injections were made at ten-day intervals. The test animal later proved to be susceptible to hog cholera.

Desiccated serum virus was tested until it was no longer infectious. A susceptible pig was likewise injected with two 10 cc. units of a 50 per cent suspension in physiologic saline solution. The test animal was later proven to be susceptible to hog cholera.

**FORMALIZED TISSUES**

Liver, spleen and brain tissues taken from cholera infected swine were finely triturated and inactivated with 1 per cent formalin. Susceptible pigs were injected with two 10 cc. units of a 50 per cent tissue suspension in a diluting agent consisting of equal parts of glycerine and physiologic saline solution. These injections were made at ten-day intervals. The test animal proved to be susceptible to hog cholera.

**PHENOLIZED TISSUE**

Tissues taken from the liver, spleen and brain of cholera infected swine were finely triturated and inactivated with 2 per cent phenol. Susceptible pigs were injected at ten day intervals with two 10 cc. units of a 50 per cent tissue suspension in a diluting agent composed of equal parts of glycerine and physiologic saline solution. These animals subsequently proved to be susceptible to hog cholera.

**SATURATION OF VIRUS WITH GASES**

Blood from cholera infected swine was diluted with equal parts of sterile distilled water and then saturated with gas. The gases used were nitrogen, chlorine, sulphur dioxide, oxygen, carbon dioxide and hydrogen. In the virulence test the pig injected with the carbon dioxide preparation contracted hog cholera. The hydrogen and oxygen dioxide preparations caused a slight temperature reaction in the susceptible pigs, but produced no symptoms. These pigs were injected with two 10 cc. units of these preparations at ten-day intervals. The immunity test, which was conducted ten days following the last injection, resulted in cholera infection in all pigs except those injected with the oxygen and hydrogen preparations.

**CONCLUSIONS**

1. The preparations made from infective tissues inactivated by desiccation apparently have no value in producing immunity against hog cholera.
2. The preparations made from fresh tissue inactivated by phenol and formalin in the concentrations used did not seem to be effective in producing any degree of immunity.
3. The saturation of blood of infected swine with certain gases inactivates the virus.
4. The preparation attenuated or inactivated by oxygen and hydrogen apparently have some value in producing immunity against hog cholera.
THE BIOCHEMISTRY OF SLUGGISH BUTYL-ACETONIC FERMENTATIONS

DONALD F. STARR

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

In a normal butyl-acetonic fermentation there are three stages: 1, there is a vigorous bacterial reproduction and acid formation; 2, the acidity decreases with the parallel production of the solvents n-butanol, acetone and ethanol, in very nearly the ratio 6:3:1; 3, the acidity rises slowly, approaching a practically constant level. Two principal types of abnormal fermentations are of special importance in the industrial process, those caused by bacterial contamination, and the sluggish fermentations characterized by a prolonged acidity peak, decreased yields of solvents, incomplete utilization of fermentable carbohydrate and a generally slow fermentation. If a normal fermentation is inoculated with a small amount of material from a sluggish culture the normal culture may become sluggish. If a sluggish culture is filtered through a bacterial filter, a very small amount of filtrate may cause a normal culture to become sluggish; this type of sluggishness can be transmitted serially by means of the use of the filtrate. The sluggish principle has some of the characteristics of bacteriophage, but experiments have so far shown no evidence of lysis. A large commercial industry suffered heavily in 1923 through what was termed as “epidemic sluggishness.” Legg patented a process for ‘immunizing’ the cultures against sluggishness.

The purpose of this thesis was three-fold:
1. To study the properties of the sluggish principle; 2. to study the biochemistry of the sluggish fermentation; 3. to study various methods for the elimination of the sluggishness.

EXPERIMENTAL

Fifteen cultures were studied as to their susceptibility to sluggishness. There was a wide variation in resistance from extremely susceptible to wholly resistant.

The toxic filtrate, containing the sluggish principle, was prepared from a culture freshly isolated from wheat. It may be stated that many trials are necessary to find a sluggish culture, indicating that the sluggish principle is not produced by the bacteria, but is occasionally associated with it in the material used for isolation. A very satisfactory filter was the Chamberlain-Pasteur filter chamber designated as L5.

Studies on the effect of pH on filterability showed values of 4.5-9.0 suitable, but the optimum range for storage was pH 5.6-6.0; filtrates more acid than pH 5.3 were badly deteriorated in potency after three months storage at room temperature.

1Original thesis submitted December, 1933.
2U. S. Patent 1,668,814. 1928.
Two strains of the bacteria were subjected to Legg’s immunization procedure, but after 17 treatments did not develop resistance. Eight cultures were subjected to a modification of the above method. One of the cultures developed a high resistance.

Studies on the effect of the filtrate on the course of the fermentation of 5 per cent corn mash showed that in the presence of the sluggish principle the bacterial counts are lower during the first part of the fermentation, but may exceed the normal counts at the end of the fermentation; the reduction of methylene blue is slower in the initial stages but more rapid in the final stages of the fermentation than normally; the production of reducing sugar is slower and does not attain as high a value as in the normal fermentation. There is no difference in the action on gelatin as measured by viscosity and formol titration. The maximum gas production occurred 15 to 20 hours later than in the normal fermentation. There is sufficient difference between the thermal death points for the bacteria and the sluggish principle to suggest that one advantage in heat shocking spores before starting a fermentation may be due to the destruction of the sluggish principle.

Dilution experiments with the toxic filtrates showed that 1,000 units per cc. could be definitely demonstrated and that over 100,000 units of the sluggish principle were present in some filtrates.
THE BUTYL-ACETONIC FERMENTATION OF THE SUGARS WITH SPECIAL REFERENCE TO XYLOSE

LELAND A. UNDERKOFLER

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

The commercial development of the butyl alcohol fermentation industry marks a big step in the utilization of agricultural products in the manufacture of industrial chemicals. This industry, just before the depression, utilized about 30,000,000 bushels of corn annually, the principal use of the butyl alcohol being in the preparation of nitrocellulose lacquers. The finish on the average automobile represents the fermentation products of one and three-quarters bushels of corn. The starch is transformed into the solvents, butanol, acetone, and ethanol in the approximate ratio of 6:3:1, together with carbon dioxide and hydrogen. The latter two products are used in the synthesis of methanol. The principal raw material is corn.

Quantitative studies on the butyl fermentation of carbohydrates other than starch, are limited. The material presented in this thesis deals with the butyl fermentation of several carbohydrates, especially xylose. This latter sugar, produced by the hydrolysis of pentosan-containing materials, is probably the cheapest carbohydrate for this process. It is obtained especially from agricultural wastes such as corn cobs, oat hulls, peanut shells, cottonseed hull bran, and straws. Due to methods developed by the United States Bureau of Standards, crystalline xylose is now economically available for quantitative studies on fermentation.

METHODS

The butyl organism was isolated from wheat and handled and stored by the usual technique. In practically all of the studies the fourth or fifth transfer from the original "soil-culture" was used for inoculation of the various media employed. The stock medium for carrying the culture was a 6 per cent corn mash. The following analyses were made at various periods of time during the fermentation: total acidity, total solvents, butanol, acetone, ethanol, volatile acids, carbon dioxide, hydrogen, and sugar unfermented. The yields were expressed in terms of the percentage of total glucose equivalent, that is, on the basis of the quantity of carbohydrate required to furnish the same amount of carbon as the glucose.

EXPERIMENTAL RESULTS

A. ACTION OF THE BUTYL ORGANISMS ON VARIOUS CARBOHYDRATES

1. Replacement of corn meal by carbohydrates and sources of nitrogen

Equivalent amounts of corn meal were replaced by starch, glucose, sucrose, and xylose. The meal could be replaced by the following per-

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1Original thesis submitted June, 1934.
centage of the carbohydrates before decreased solvent yield was apparent: starch, 90 per cent; glucose or sucrose, 80 per cent; and xylose, 50 per cent.

Various sources of nitrogen were used in place of the corn meal, employing xylose as the substrate. These included peptone, tankage, steep water, corn-gluten meal, casein, and ammonium chloride. The ammonium chloride and steep water gave the poorest results; the best yields were obtained with the peptone and corn-gluten meal. The highest yields with the gluten meal were obtained in the medium containing 0.5 gram of the material per 100 cc. The addition of varying amounts of K₂HPO₄, MnSO₄, NaCl, MgSO₄, or FeSO₄ did not increase the yields.

2. The course of the butyl-acetonic fermentation of various carbohydrates

The medium contained 0.5 per cent corn-gluten meal together with the carbohydrates tested. These included starch, glucose, maltose, levulose, sucrose and xylose. At various periods of time determinations were made of the total acidity, pH, butanol, acetone, ethanol, total solvents, and sugar consumed. All of the sugars were attacked by the butyl organisms and at maximum solvent yields the ratio of solvents produced was that normal for corn mash, that is, about 6:3:1. In general, the fermentations of the carbohydrates in the semi-synthetic medium were marked by greater time required, less pronounced acidity breaks, and somewhat poorer utilization of carbohydrates. The maximum total solvent yield showed little variation from that of corn mash.

A careful fractional analysis of the distillate showed that butanol, acetone, and ethanol are the only neutral volatile products formed in appreciable amounts.

B. INFLUENCE OF VARIOUS FACTORS UPON SOLVENT YIELD

1. Influence of surface-volume ratio

It has often been observed in practice that larger yields may be obtained in a large-scale fermentation than on a laboratory scale, a fact which might reasonably be associated with surface-volume ratio. The results of experiments to test this theory are given in table 1, from which it is evident that the solvent production does increase with decrease in surface-volume ratio.

<table>
<thead>
<tr>
<th>Flask size (cc.)</th>
<th>Vol. of medium (cc.)</th>
<th>Surface (sq. cm.)</th>
<th>Surface-volume ratio*</th>
<th>Yield, percentage of glucose equiv.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Corn</td>
</tr>
<tr>
<td>150</td>
<td>100</td>
<td>22.5</td>
<td>0.225</td>
<td>20.58</td>
</tr>
<tr>
<td>500</td>
<td>300</td>
<td>50</td>
<td>0.167</td>
<td>24.66</td>
</tr>
<tr>
<td>1,000</td>
<td>750</td>
<td>62</td>
<td>0.085</td>
<td>27.10</td>
</tr>
<tr>
<td>2,000</td>
<td>1,500</td>
<td>80</td>
<td>0.055</td>
<td>28.66</td>
</tr>
<tr>
<td>4,000</td>
<td>3,000</td>
<td>110</td>
<td>0.037</td>
<td>30.24</td>
</tr>
</tbody>
</table>
2. Prolonged incubation

It has been found in practice that after active fermentation has stopped the solvents decrease in amount, with accompanying rise in acidity, hence the solvents are distilled before this change takes place. It was found that the same phenomenon occurs in the fermentation of xylose in the semi-synthetic medium, as shown by the data in table 2.

TABLE 2. Solvent yields from xylose

<table>
<thead>
<tr>
<th>Time in days</th>
<th>Acidity $^1$</th>
<th>Solvent yield $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.50</td>
<td>......</td>
</tr>
<tr>
<td>1</td>
<td>4.37</td>
<td>6.41</td>
</tr>
<tr>
<td>2</td>
<td>2.57</td>
<td>16.41</td>
</tr>
<tr>
<td>3</td>
<td>2.35</td>
<td>25.00</td>
</tr>
<tr>
<td>4</td>
<td>2.35</td>
<td>27.41</td>
</tr>
<tr>
<td>5</td>
<td>2.45</td>
<td>27.08</td>
</tr>
<tr>
<td>6</td>
<td>2.87</td>
<td>25.90</td>
</tr>
<tr>
<td>7</td>
<td>3.10</td>
<td>25.30</td>
</tr>
<tr>
<td>8</td>
<td>2.97</td>
<td>24.76</td>
</tr>
<tr>
<td>9</td>
<td>3.35</td>
<td>24.08</td>
</tr>
<tr>
<td>10</td>
<td>3.35</td>
<td>23.38</td>
</tr>
<tr>
<td>11</td>
<td>3.40</td>
<td>23.38</td>
</tr>
<tr>
<td>12</td>
<td>3.35</td>
<td>22.29</td>
</tr>
<tr>
<td>13</td>
<td>3.40</td>
<td>21.48</td>
</tr>
</tbody>
</table>

$^1$Cc. of 0.1 N NaOH required for 10 cc. of medium.

$^2$Yield of total solvents, percentage of glucose equivalent.

3. Inoculation from different transfers

It is common practice to use the fourth or fifth transfer to inoculate the mash for the production of solvents. Experiments were conducted to determine the influence of the number of transfers upon the solvent yield from xylose. The best yields were obtained by using from the second to the seventh transfer. The yields were materially less on the eighth and ninth transfers.

4. The continuous carbon balance

The course of the butyl-acetonic fermentation of xylose was determined with reference to the following items: total acidity, pH, xylose fermented, butanol, acetone, ethanol, carbon dioxide, butyric acid, acetic acid, and non-volatile acid. Data are given in table 3 for the carbon balance at various periods of time.
It is evident that the carbon recovered in the products during the early stages of the fermentation was considerably greater than the sugar consumed. This discrepancy may be associated with intermediates which also reduced the copper solution in the sugar analysis. After the fermentation had attained normal maximum yields the carbon balance approached the theoretical very closely.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Carbon in xylose (gram-atoms)</th>
<th>Carbon in products (gram-atoms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>5.0</td>
<td>6.21</td>
</tr>
<tr>
<td>60</td>
<td>5.0</td>
<td>5.74</td>
</tr>
<tr>
<td>68</td>
<td>5.0</td>
<td>5.50</td>
</tr>
<tr>
<td>80</td>
<td>5.0</td>
<td>4.88</td>
</tr>
<tr>
<td>92</td>
<td>5.0</td>
<td>4.82</td>
</tr>
<tr>
<td>122</td>
<td>5.0</td>
<td>4.88</td>
</tr>
<tr>
<td>144</td>
<td>5.0</td>
<td>5.06</td>
</tr>
</tbody>
</table>
THE PRODUCTION OF ALCOHOLS BY THERMOPHILIC FERMENTATIONS

MATTHEW KERMIT VELDHUIS

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

Cellulose and cellulosic materials constitute a large proportion of such products as corn cobs, oat hulls, beet pulp, peanut hulls and the straws. One method for the utilization of these materials is their conversion into industrial chemicals by means of fermentation. This may be accomplished by direct bacterial action upon the cellulosic materials, or by the action of the appropriate microorganisms upon the hydrolyzed material. The most promising type of direct fermentation is that brought about at high temperatures, that is, the thermophilic fermentation. Two commercial developments have taken place in this field; the first, the production of combustible gases, and the second, the production of acetic acid.

A great deal of work has been done in the thermophilic fermentation of cellulose; most of the research has been directed toward the production of high yields of acetic acid and the isolation of pure cultures of the bacteria involved. Yields of acetic acid as high as 50 per cent of the cellulose added have been reported. A survey of the literature shows an occasional report of the production of ethyl alcohol by the thermophilic fermentation of cellulose, but little systematic work has been done with particular reference to the maximum production of this chemical. The purpose of this thesis was to determine the optimum conditions for the production of maximum yields of ethyl alcohol by the thermophilic fermentation of cellulose.

The cultures were obtained from several sources and were selected on the basis of rate of growth and ability to produce alcohols and volatile acids. It was noticed that some cultures gave relatively high alcohol yields while others gave relatively high yields of volatile acids. The cultures which produced the highest yields of alcohol came from a compost pile and a mixture of rotting horse manure and straw.

The ethanol and butanol were separated from the fermented liquor by neutral distillation, and were estimated by a wet oxidation method, using potassium dichromate. The volatile salts were separated by the distillation of an acidified sample, estimated by titration with standard hydroxide, and calculated as acetic acid. The ratios of acetic and butyric acid were determined by the method of Fyleman. Non-volatile acids were separated by the extraction with ether of the residual liquor from the volatile acid distillation, estimated by titration with sodium hydroxide, and calculated as lactic acid. Reducing sugars were determined by the method of Shaffer and Hartmann. The gaseous products were analyzed with a Williams gas analysis apparatus. The amount of residual cellulose was determined by treating a sample of the fermented liquor...
with hydrochloric acid, filtering, treating the residue with a hot sodium hydroxide solution, washing, drying and weighing.

Using media consisting of filter paper, ammonium chloride, di-potassium phosphate, excess calcium carbonate, and tap water, fermentations were conducted at temperatures ranging from 37.5° C. to 65° C. At 37.5° C. no growth was evident even after 50 days. The highest yield of alcohols was obtained at 55° C., and the maximum yield was reached in 8 days. The highest yield of acetic acid, on the other hand, was obtained at 60° C., and the maximum yield was reached in 11 days.

Systematic studies were made of the effect of the composition of the media upon the production of ethyl alcohol. The cultures had been grown for some time on media consisting of filter paper, ammonium chloride, di-potassium phosphate, and excess calcium carbonate in tap water, but it was not known whether the optimum concentrations were being used. When the concentration of ammonium chloride was varied, the largest yields of alcohols were obtained within the range of 0.20 to 0.55 g. of ammonium chloride (NH₄Cl) per 100 cc. When the concentration of di-potassium phosphate was varied, the yields of alcohols were greatest within the range of 0.20 to 0.40 g. of di-potassium phosphate (K₂HPO₄·3H₂O) per 100 cc. With variation in concentration of cellulose, little change in the efficiency of the conversion of cellulose to alcohols was noticed within the range of 2 to 5 g. of cellulose per 100 cc.

At the conclusion of these experiments, a medium consisting of 3 g. of cellulose, 0.25 g. of ammonium chloride, and 0.25 g. of di-potassium phosphate per 100 cc. of tap water was adopted.

In most of the experiments calcium carbonate had been used as the neutralizing agent to react with the acids formed and regulate the pH; the use of calcium carbonate gave a pH value of 6.5 to 6.8. In order to determine the effect of pH, fermentations were adjusted twice daily with sodium hydroxide or sodium carbonate to pH values ranging from 5 to 9. The highest yields of alcohols were obtained when the adjustments were made to pH values of 7.5 to 8.0. Thus it was shown that the calcium carbonate had not been giving a reaction sufficiently alkaline to give maximum yields of the alcohols.

In further attempts to increase the yields of alcohols, it was found that aeration decreased the yield, and that the addition of 0.50 g. of peptone per 100 cc. increased the yield 28 per cent. The addition of glucose up to 0.25 g. per 100 cc. increased the yield of alcohols slightly, while larger amounts of glucose were inhibitory to the fermentation of cellulose. The presence of glucose did not increase the rate of decomposition of the cellulose. Continued growth on a glucose medium containing no cellulose caused the culture to lose its cellulose-fermenting power.

The alcohols produced during the fermentation were shown to be ethanol and n-butanol, in the ratio of about 20 to 1. The volatile acids consisted of acetic acid and butyric acid in the ratio of about 20 to 1. The gases were found to consist mainly of carbon dioxide and hydrogen. Sometimes very small amounts of methane were also formed. The percentage of hydrogen in the gases was greatest during the early stages of the fermentation. By using the best alcohol producing cultures in the synthetic medium described above, yields of 16 to 18 per cent, based on the cellulose added, were obtained quite regularly at 55° C. By adjusting
medium to a pH value of 7.5, the yields were increased to values of 20 to 25 per cent. A fermentation at 60° C., under otherwise optimum conditions, gave 26.6 per cent carbon dioxide; 24.4 per cent volatile acids, calculated as acetic acid; 26.3 per cent ethanol; 0.715 per cent butanol; 0.71 per cent reducing sugar; 0.356 per cent hydrogen; 0.244 per cent non-volatile acids, calculated as lactic acid; 0.027 per cent methane; 12.85 per cent of a material soluble in acids and insoluble in bases; and 3.22 per cent of unfermented cellulose. The total recovery was thus 95.422 per cent by weight.
THE ALKALINE OXIDATION OF LIGNIN

ARTHUR WILLIAM WALSE

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

The purpose of the thesis centers around the study of the method of preparation of lignin from cornstalks and oat hulls by the use of aqueous ammonia, and the study of the oxidation of lignin with an alkaline iodine solution.

A short historical review on the general subject of lignin, together with several definitions of lignin, are given. The indefiniteness of the term is indicated.

The more general methods for the preparation of lignin are reviewed, including the acid, sulfite, alkali and neutral solvent methods. The non-uniformity of the lignin preparations are compared with one another, and the question of purity is emphasized.

The more important formulae presented in the literature for the possible structure of lignin are given. These formulae include those of Schrauth, Freudenberg, Doré and Hall, Cross and Bevan, Fuchs, and Klason.

A short review of the literature bearing directly on the experimental procedures is given. The various oxidation products of lignin obtained by different methods of preparing and oxidizing alkali lignins, and those obtained by different investigators, are listed. The importance of the results is discussed.

Ammonia lignin was prepared from cornstalks by the following procedure: The tissues were cooked at 100 pounds pressure for eight hours with concentrated aqueous ammonia. The lignin and pentosans were precipitated with acid. The pentosans were removed by dilute acid hydrolysis. Yields, properties and the objections to the method were discussed.

A new method for preparation of alkali lignin from oat hulls was investigated. The method was carried out essentially the same as that for cornstalks, except that the hydrolysis of the pentosans preceded the alkaline extraction with ammonia.

The oat hull lignin was oxidized with alkaline hypohalite. Use of an alkaline solution of chlorine was found to give indefinite products. The same result occurred with alkaline bromine solution, but crystalline carbon tetrabromide was characterized and identified as one of the oxidation products.

Alkaline solutions of iodine were found to react quantitatively with lignin. The effect of time on the iodine equivalent was studied; oxidation was complete and remained constant after thirty-six minutes. Iodoform was proven to be one of the oxidation products.

Lignin oxidized by alkaline iodine was prepared and studied. The methoxyl content was found to be 7.2 to 7.4 per cent and the ash content

*Original thesis submitted December, 1933.
0.5 per cent. A comparative table of the properties of the oxidized and the unoxidized lignin is given. Methylated lignin was found not to oxidize in an alkaline iodine solution. Nitrolignin could not be oxidized quantitatively.

Acid hydrolysis did not appreciably affect the iodine equivalent of oat hull lignin. Sugars could not be identified in the solution.

Oat hull lignin was oxidized by Fehling’s solution. Analysis of the copper reduced corresponded to a dextrose equivalent of 5.4 per cent. The change in the iodine equivalent was 34 cc. of 0.1 N iodine per gram sample.

Sulfuric acid lignins from spruce wood, aspen wood and oat hulls were found to have iodine equivalents of 68, 112 and 160 cc. of 0.1 N iodine solution per gram sample, respectively. Iodoform was present in the oxidized solution of each.

Oxidized oat hull lignin containing 7.23 per cent of methoxyl was methylated with dimethyl sulfate. The product contained 22.19 per cent of methoxyl and was found to be insoluble in cold sodium hydroxide solution. When the methylated product, suspended in 10 per cent sodium hydroxide solution, was warmed to approximately 80° C. it began to go into solution, and at 90° C. was completely soluble in the alkali. The alkali-soluble product was reprecipitated with dilute sulfuric acid and was found to contain 17.4 per cent of methoxyl and 10.5 per cent iodine.

Five different tissues were oxidized with an alkaline iodine solution both before and after hydrolysis. The samples were dried at 105° C. for two hours. The acid hydrolysis was made with 0.1 N. hydrochloric acid at ten pounds pressure for six hours. The time of oxidation was increased to four hours and the end point in the titration was considered to be the point at which the starch solution remained colorless for five minutes. The lignin was determined at 4° C. by the 72 per cent sulfuric acid method; the methoxy content was determined by the Zeisel method. The oxidation values were based on the dry weight of the material analyzed. The results are given in the following table.

The significance of the alkaline oxidation of lignin is emphasized in the discussion of the results. The formation of iodoform during oxidation indicates the presence of a methyl carbinol or a methyl ketonic group in lignin. It is emphasized that the same methoxy contents were obtained from lignin prepared from different ammonia cooks. The methoxy contents of oxidized lignin were the same from the cold alkali extraction of acid hydrolyzed hulls as that from the ammonia cooking process. The iodine equivalent of the isolated lignin was of the same order of magnitude as that calculated for the lignin present.
## ALKALINE OXIDATION OF LIGNIN

207

### TABLE 1. The effect of acid hydrolysis on the alkaline-iodine oxidation equivalent, lignin content and methoxy content of several plant tissues

<table>
<thead>
<tr>
<th>Original Tissue</th>
<th>Oat hull percentage</th>
<th>Rye straw percentage</th>
<th>Flax straw percentage</th>
<th>Artichoke stalks percentage</th>
<th>Corn stalks percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>19.07</td>
<td>21.04</td>
<td>22.87</td>
<td>23.99</td>
<td>24.74</td>
</tr>
<tr>
<td>Methoxy</td>
<td>2.62</td>
<td>3.51</td>
<td>3.47</td>
<td>4.51</td>
<td>3.11</td>
</tr>
<tr>
<td>Calc. methoxy in lignin</td>
<td>13.74</td>
<td>16.68</td>
<td>15.17</td>
<td>18.80</td>
<td>12.57</td>
</tr>
<tr>
<td>N/10 iodine equivalent, cc./gram sample</td>
<td>16.56</td>
<td>27.58</td>
<td>17.90</td>
<td>18.42</td>
<td>39.90</td>
</tr>
<tr>
<td>N/10 iodine equivalent, cc./gram lignin</td>
<td>86.80</td>
<td>131.10</td>
<td>78.30</td>
<td>76.80</td>
<td>161.30</td>
</tr>
</tbody>
</table>

HYDROLYZED TISSUE

<table>
<thead>
<tr>
<th>Original Tissue</th>
<th>Oat hull percentage</th>
<th>Rye straw percentage</th>
<th>Flax straw percentage</th>
<th>Artichoke stalks percentage</th>
<th>Corn stalks percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>27.46</td>
<td>33.86</td>
<td>34.53</td>
<td>37.23</td>
<td>43.79</td>
</tr>
<tr>
<td>Methoxy</td>
<td>3.77</td>
<td>4.92</td>
<td>4.82</td>
<td>6.01</td>
<td>4.34</td>
</tr>
<tr>
<td>N/10 iodine, cc./gram sample</td>
<td>47.71</td>
<td>57.11</td>
<td>35.52</td>
<td>51.85</td>
<td>71.50</td>
</tr>
<tr>
<td>N/10 iodine, cc./gram lignin</td>
<td>171.20</td>
<td>168.60</td>
<td>103.90</td>
<td>139.20</td>
<td>163.30</td>
</tr>
</tbody>
</table>

b. Calc. on basis of original tissue—

<table>
<thead>
<tr>
<th>Original Tissue</th>
<th>Loss due to hydrolysis</th>
<th>Fraction of total hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>14.43</td>
<td>24.33</td>
</tr>
<tr>
<td>Methoxy</td>
<td>1.98</td>
<td>24.40</td>
</tr>
</tbody>
</table>

### LOSS DUE TO HYDROLYSIS

<table>
<thead>
<tr>
<th>Original Tissue</th>
<th>Loss due to hydrolysis</th>
<th>Fraction of total hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>4.64</td>
<td>24.33</td>
</tr>
<tr>
<td>Methoxy</td>
<td>0.64</td>
<td>24.40</td>
</tr>
<tr>
<td>Methoxy in lignin</td>
<td>0.01</td>
<td>24.40</td>
</tr>
</tbody>
</table>

FRACTION OF TOTAL HYDROLYZED

<table>
<thead>
<tr>
<th>Original Tissue</th>
<th>Loss due to hydrolysis</th>
<th>Fraction of total hydrolyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin</td>
<td>24.33</td>
<td>24.33</td>
</tr>
<tr>
<td>Methoxy</td>
<td>24.40</td>
<td>24.40</td>
</tr>
</tbody>
</table>
STUDIES ON THE ESCHERICHIA-AEROBACTER GROUP OF BACTERIA IN DAIRY PRODUCTS

MAURICE WADE YALE

From the Department of Dairy Industry, Iowa State College

Accepted for publication August 1, 1934

The Escherichia-Aerobacter group of bacteria was studied on a species basis with respect to numbers in dairy products, development of off-flavors in experimental butter and action on milk constituents.

Bergey's classification of the group was revised to agree with original descriptions and Escherichia foetida (Perez), E. nocticarii (White), E. sphingidis (White), E. ichthyosmia (Hammer), E. iliaca (Ford) and Aerobacter bombicus (Glaser) were dropped from the group because original descriptions stated that they did not ferment lactose. E. schaefferi (von Freudenreich) was considered synonymous with E. coli (Escherich).

Two hundred and four cultures belonging to the Escherichia-Aerobacter group were isolated from 212 samples of dairy products as follows: 91 cultures from 70 samples of raw milk; 21 from 64 samples of pasteurized milk; 42 from 24 samples of raw cream; 16 from 20 samples of ice cream; 9 from 9 samples of ropy milk and cream; and 25 from 25 samples of defective butter.

The genus Escherichia comprised 63 per cent of the cultures from raw milk; 57 per cent from pasteurized milk; 33 per cent from raw cream and 31 per cent from ice cream.

The genus Aerobacter comprised 26 per cent of the cultures from raw milk; 10 per cent from pasteurized milk; 56 per cent from raw cream; 56 per cent from ice cream; 100 per cent from ropy milk and cream; and 88 per cent from defective butter.

Ten of 29 species included by Bergey in the genus Escherichia were identified, viz., E. coli, E. pseudocoloides, E. communior, E. paragrünthali, E. vesiculiformans, E. formica, E. enterica, E. anaerogenes, E. grünthali and E. neapolitana. Three of 6 species included by Bergey in the genus Aerobacter were identified, viz., A. aerogenes, A. cloacae and A. oxytocum. E. coli was the most common species in raw milk; E. pseudocoloides in pasteurized milk; A. cloacae in ice cream; and A. aerogenes in raw cream, ropy milk and cream, and defective butter. Of 80 cultures that could not be identified on the basis of species in Bergey's classification 26 belonged to an intermediate group.

While 0.01 cc. quantities of raw milk from composite supplies usually showed the presence of organisms of the Escherichia-Aerobacter group, 10 cc. quantities of pasteurized milk taken from the pasteurizer showed their presence in only 1 out of 15 pasteurizations run at the College Dairy and in only 5 out of 17 runs at 13 other Iowa plants. In the latter case, 3 positive samples were known to have been improperly pasteurized.

*Original thesis submitted June, 1931.
At the College Dairy, organisms of the Escherichia-Aerobacter group were present in a larger percentage of cases and in higher numbers in the first milk bottled than in milk bottled at a later stage. The presence of the highest numbers of these organisms in the first milk bottled indicated that contamination from equipment was gradually reduced by the flow of the pasteurized milk. Similar results were obtained at the other pasteurization plants studied.

Both young and old cultures of *E. paragrünthali* and *A. cloacae* that were originally isolated from pasteurized milk were destroyed by 20 minutes heating at 62° C. (143.6° F.). Old cultures were slightly more resistant than young cultures.

The number of Escherichia-Aerobacter organisms in 24 samples of cream for butter making ranged between 25 per cc. and 600,000 per cc. The counts exceeded 10,000 per cc. in over 60 per cent of the samples. In 20 samples of ice cream from 11 commercial plants the number of these organisms ranged between 3 per cc. and 2,500 per cc. and exceeded 100 per cc. in 30 per cent of the cases.

Of 25 cultures isolated from 17 samples of off-flavored butter, 15 were identified as *A. aerogenes*, 4 as *A. cloacae*, 3 as *A. oxytocum* and 3 as belonging to an intermediate group.

Flasks of pasteurized cream were inoculated with cultures of *E. coli*, *E. communior*, *E. formico*, *E. paragrünthali*, *A. aerogenes*, *A. oxytocum* and *A. cloacae*. Churnings of butter were divided into two lots, one of which was salted. Each lot was divided into two portions, one of which was held at about 7° C., and the other at about 18° C.

In 10 days at about 7° C., species of the genus Escherichia did not grow in salted butter, but in unsalted butter some of them did, while species of the genus Aerobacter sometimes grew in the salted butter and regularly grew in the unsalted.

In 10 days at about 18° C., species of both genera grew in salted as well as unsalted butter, but those of the genus Aerobacter grew more rapidly and reached higher numbers than did those of the genus Escherichia.

In 10 days at about 7° C., species of the genus Escherichia did not develop off-odors and flavors in butter, either when salted or unsalted; species of the genus Aerobacter did not develop off-odors and flavors in salted butter, but sometimes did in unsalted butter.

In 10 days at about 18° C., species of the genus Escherichia did not develop off-odors and flavors in butter either when salted or unsalted; species of the genus Aerobacter regularly developed off-odors and flavors in either salted or unsalted butter.

The unclean odor and flavor produced in butter by species of the genus Aerobacter definitely resembled the condition produced in milk by these organisms.

*E. coli*, *E. communior*, *E. formica*, *A. aerogenes* and *A. oxytocum* did not attack butter fat or cause appreciable proteolysis.

*E. coli*, *E. communior* and *E. formica* produced volatile acidities ranging from 68.0 to 80.5 (number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1,200 gram portion of fermented milk), while *A. aerogenes* and *A. oxytocum* produced volatile acidities ranging from 25.9 to 48.0. With the addition of 0.2 and 0.4 per
citric acid the species belonging to the genus Escherichia produced increased volatile acidities, while the species belonging to the genus Aerobacter in most cases showed a slight decrease. The volatile acids produced by species of the genus Escherichia were largely acetic with small amounts of propionic as determined by the percentages of barium in the barium salts while they were entirely acetic as determined by Duclaux values.
SOME QUANTITATIVE STUDIES OF ORGANOMETALLIC COMPOUNDS

ERWIN A. ZOELLNER

From the Department of Chemistry, Iowa State College

Accepted for publication August 1, 1934

This thesis has for its purpose the consideration of: First, a survey of the available literature on the comparative utility of the RMgCl, RMgBr and RMgI compounds in the practical application of the Grignard reagents; second, a study of the preparation of a number of organomagnesium chlorides, bromides and iodides, under general or special conditions; and third, the preparation of methyl-lithium from methyl chloride and n-butyl-lithium from n-butyl iodide.

The survey of the available literature, on the comparative utility of the RMgCl, RMgBr and RMgI compounds shows, in general, that the best results are obtained with the RMgCl and RMgBr compounds, and of these two, the RMgCl compounds are somewhat superior.

Methyl- and ethylmagnesium chlorides have been prepared from the gaseous halides in a closed system and under a pressure of 50 mm. Otherwise, the apparatus was that generally used for the preparation of Grignard reagents. The yields of the above Grignard reagents were 84.0 and 90.3 per cent, respectively.

Studies have been made of several alkylmagnesium chlorides in the same apparatus and under the same two conditions previously described. In these studies, 0.05 mole of halide was reacted with 0.055 atom of commercial magnesium turnings. Under the first condition, the halides were added to the magnesium in ether over a period of thirty to thirty-five minutes and under the second condition, the halides were added all at once. The yields of the RMgCl compounds are given below with hydrocarbon radicals of the Grignard reagents (yields under the second condition are given in parenthesis): n-Propyl, 87.5; iso-propyl, 80.0; n-butyl, 91.9 (91.7); iso-butyl, 84.0 (77.2); sec-butyl, 90.1 (88.2); tert-butyl, 45.0 (40.0); a-sec-hexyl, 89.2 (83.2); β-sec-hexyl, 102.0 (96.7); β-phenylthyl, 88.9 (87.9); γ-phenylpropyl, 87.2 (85.3 per cent). When the dropping funnel and condenser were kept cold, the yield of iso-propylmagnesium chloride was 88.8 per cent.

A higher concentration of the alkyl chlorides in ether was necessary to start the reactions with magnesium in the usual manner, than that required for the corresponding alkyl bromides and iodides. Since the alkyl chlorides reacted slower than the corresponding alkyl bromides and iodides with magnesium, it took approximately an hour longer to carry out the reactions of the alkylmagnesium chlorides than the corresponding RMgBr and RMgI compounds.

1Original thesis submitted August, 1933.
In view of the data given above on the survey of the comparative utility of the RMgCl, RMgBr and RMgI compounds and in consideration of the generally superior yields of the alkylmagnesium chlorides over the corresponding RMgBr and RMgI compounds, it is advisable to use the RMgCl compounds in the practical application of the Grignard reagents whenever these can be conveniently prepared.

Allylmagnesium chloride was prepared, under the same conditions used in this laboratory for the preparation of the corresponding RMgBr and in the same yield (90 per cent), by slow addition of the halide to excess of 30-80 mesh magnesium powder. More than 75 per cent of the runs were lost, however, due to the fact that the reaction mixtures became colloidal. Since this difficulty has, so far, not occurred in the preparation of allylmagnesium bromide, it is not now advised to replace the RMgBr by the RMgCl compound.

Studies have been made of the preparation of a number of organomagnesium bromides under the conditions of the alkylmagnesium chlorides given above. The yields of these organomagnesium bromides are also given in the same manner: Lauryl, 85.3 (74.3); cetyl, 72.6-79.5; β-phenylethyl, 89.3 (82.7); 2-cymyl, 87.0 (82.7); α-methoxyphenyl, 99.3 (96.3); p-methoxyphenyl, 91.2 (82.5); o-ethoxyphenyl, 98.8 (98.5); p-ethoxyphenyl, 84.5 (78.5); p-bromophenyl, 103.3 (99.8); p-chlorophenyl, 95.8 (91.2); β-styryl, 50 (42 per cent).

It was found that p-diphenyl- and mesitylmagnesium bromide could be best prepared in 0.05 mole runs with commercial magnesium turnings, by adding all the halide to the magnesium at once. In this manner, the p-diphenylmagnesium bromide was prepared in a six-hour period of refluxing and stirring in yields of 75 to 77 per cent, and of mesitylmagnesium bromide in yields of 82 to 83 per cent in a three hour period of refluxing. The yields were not improved by the use of fine magnesium. It was found difficult to start the reaction of mesitylmagnesium bromide without the use of activated 12.75 per cent copper-magnesium alloy. With this activator the reaction started immediately and the yields were slightly improved.

Under comparable conditions, tert-butylmagnesium chloride, bromide and iodide were obtained in yields of 83; 66.6 and 56.0 per cent, respectively, by slow addition of the halides to three equivalents of 30-80 mesh magnesium powder. Under the above conditions, the yield of β-styrylmagnesium bromide was 85 to 90 per cent. When three equivalents of ordinary magnesium turnings were used, the yield was 75 to 85 per cent.

Studies have been made of several organomagnesium iodides under the same conditions used for alkylmagnesium chlorides. The yields are also given in the same manner: Methyl, 90.3 (90.1); ethyl, 87.5 (78.5); n-propyl, 80.7 (71.4); n-butyl, 80.1 (62.0); n-amyl, 78.2 (58.2); n-nonyl, 66.2-70.1; n-decyl, 51.1-58.8; n-undecyl, 69.5-71.5; cetyl, 69-77; phenyl, 85.6 (72.3); 2-furyl, 87.9 (86.6); 2-methyl-5-furyl, 89.9 (91.3); 2-thiényl, 98.6 (99.7 per cent).

Methyl-lithium was prepared under the conditions and in the apparatus used for the preparation of methyl- and ethyl-magnesium chlorides, in a yield of 88.0 per cent, based on the halide used, and 89 per cent based on the lithium used. n-Butyl-lithium could not be prepared from n-butyl iodide.
Commemorating Six Decades of the Modern Era in Botanical Science

November 15 and 16, 1934

The era is marked by the beginning of modern teaching and research in the botanical sciences. Following the brilliant contributions of de Bary, Darwin, Pasteur, Max Schultze, Sachs, and Asa Gray, during the fifties and sixties of the last century teaching received new impetus through the introduction into the laboratory of the compound microscope. Research was freed from the fetters of biased thinking. Modern concepts began to yield new knowledge and to give birth to new sciences.

Department of Botany, Iowa State College, Cooperating with the Corn Research Institute of the Iowa Agricultural Experiment Station
Ames, Iowa

[215]
Charles Edwin Bessey and His Microscope

The teaching of botany received strong impetus through the labors of Doctor Charles Edwin Bessey, who served Iowa State College from 1870 to 1884. Probably his greatest achievement in teaching was the stimulus he gave to the initiation of modern laboratory work when he introduced the use of the compound microscope.

This compound microscope, made by Beck and Son, England, was purchased for Doctor Bessey at a cost of $1,200.00. In 1882 when the building housing the botanical laboratories was partially demolished by a cyclone, the microscope was rescued from the wreckage by Vice President Herman Knapp, then a junior student. Doctor Bessey's first concern for his laboratory after the storm was this much cherished microscope. The instrument was highly prized for a long time as the most valuable piece of technical apparatus owned by the college. All students in his classes learned of this microscope, which Doctor Bessey used in his teaching and research while he served Iowa State College.
1. Symposium: Teaching General Botany

If it is agreed that the largest responsibility of botanists to their science and to society is in the field of teaching in its broadest sense, then it is fitting and timely that we gather around the conference table and weigh the place of the old in the light of the new. To this end this symposium has been arranged and it is hoped that through critical constructive thinking we may be able to reflect new light on some of the human relations of plants.

CONTENTS

Commemorating Six Decades of the Modern Era in Botanical Science. I. E. Melhus ................................................................. 221
Teaching General Botany. Charles E. Friley ........................................... 225
The Teaching of Botany Sixty-five Years Ago. Ernst A. Bessey............. 227
The Evolution and Differentiation of Laboratory Teaching in the Botanical Sciences. R. J. Pool ....................................................... 235
The Place of Botany in a Liberal Education. Edmund W. Sinnott........... 243
The Problems in an Elementary Botany Course in a State University. George S. Bryan ................................................................. 249
Content of General Botany Courses. Homer C. Sampson....................... 253
The Development of the Group Conference System of Teaching. S. M. Dietz .................................................................................. 259
Attainments in Teaching. W. H. Lancelot .............................................. 281
Changing Emphasis in General Botany and Its Significance. Chas. F. Hottes ................................................................. 287
Botany Notebooks. H. W. Rickett ......................................................... 291
An Applied Course in General Botany. L. W. Durrell ......................... 299
A Use of the Placement Test in the Teaching of General Botany. Wm. A. Kreutzer ................................................................. 303

[219]
Author Index

Aikman, J. M., 379
Andre, Floyd, 73
Bennett, Logan J., 609
Bessey, Ernst A., 227
Bigger, J. H., 413
Bowie, Robert M., 131
Brown, F. E., 89
Brins, Richard Wedge, 133
Bryan, George S., 249
Bryner, Loren C., 137
Buchanan, R. E., 411
Calloway, Nathaniel Oglesby, 141
Charlton, D. B., 1
Conard, Henry S., 347
Davis, Glen N., 505
Decker, George C., 567
Derby, Herbert Andrew, 145
Dietz, S. M., 259
Dodge, A. F., 399
Dungan, G. H., 413
Durrell, L. W., 299
Eisele, Harold F., 521
Elliot, Charlotte, 461
Ellisor, L. O., 25
Errington, Paul L., 625
Erwin, A. T., 661
Feaster, James Floyd, 147
Flint, W. F., 413
Friley, Charles E., 225
Golding, Norman S., 151
Griffitts, F. A., 89
Haber, E. S., 61
Hall, Phoebe R., 115
Hammer, B. W., 128
Harriman, L. A., 155
Harrison, J. William, 37
Harris, William Donald, 159
Hellwig, Arthur Paul, 163
Hershey, Arthur L., 489
Hoelscher, Arnold F., 165
Hollbert, J. R., 413
Holl, D. L., 397
Hamerstrom, Jr., F. N., 625
Hottes, C. F., 287
Hughes, H. D., 559
Humphrey, L. M., 549
Jenkins, Merle T., 429
Keil, Havard Lawrence, 169
Kendall, Anson R., 171
Kreutzer, William A., 303
Lancelot, W. H., 281
Lane, C. Bronson, 173
Larson, J. A., 365
Lindstrom, E. W., 451
Loomis, W. E., 509
Lowdermilk, W. C., 337
MacDonald, Margaret B., 587
McGovran, Edward Rawson, 177
McNew, George L., 481
Martin, John N., 489
Melhus, I. E., 221
Merchant, Ival Arthur, 181
Naftziger, Theodore R., 183
Nelson, M. E., 1
Olson, H. C., 125
Plagge, H. H., 95
Pool, R. J., 235
Reddy, Chas. S., 527
Rickett, H. W., 291
Sampson, Homer C., 253
Schwarte, Louis Harold, 187
Starr, Donald F., 195
Shantz, H. L., 353
Shimek, B., 325
Simonds, Austin O., 641
Sinnott, Edmund W., 243
Sokoloff, B. D., 581
Spawn, Gerald B., 617
Stadler, L. J., 427
Tate, H. D., 677
Tauber, Oscar E., 13
Uhland, R. E., 329
Underkoffer, Leland A., 197
Veldhuis, Matthew Kermit, 201
Walde, Arthur William, 205
Wallace, H. A., 361
Wellhausen, E. J., 539
Werkman, C. H., 1
Whitlock, J. H., 667
Wilkins, F. S., 391
Yakimoff, W. L., 581
Yale, Maurice Wade, 209
Yeager, J. Franklin, 13
Zoellner, Erwin A., 213

[685]
Subject Index

Achotodes zeae (Harris), 567
Achromobacter putrefaciens, isolated from surface taint butter, 145
Acid production in butter cultures, 155
Acre yields of crosses in maize, 433, 434
Acititis macularia, 610, 619
Aerobacter oxytoca, 173
Alcohol insoluble residues from samples of apples, 110
Alcohols, means of increasing yield of, 202
production of, by thermophilic fermentations, 201
Alegria—a popping seed used in Mexico as a substitute for popcorn (Amaranthus caudatus L. var. leucospermus Th.), 661
as ancient food plant of Indian, 661
occurrence of, 662
Alfalfa, external structures of, 650
histological studies of the development of the root and crown of, 641
Alkaline oxidation of lignin, the, 205
Alternaria, growth indices for species of, 45
Amaranthus caudatus L. var. leucospermus Th., description of, 661
Amblytes jucundus (Brulle), 573
A. laetius (Brulle), 573
American golden plover (Pluvialis dominica dominica), 610, 618
American knot (Calidris canutus rufus), 611
American woodcock (Philohela minor), 619
American woodcock (Philohela minor), 619
Anatomical study of eye defect of guinea pig, 667
Anatomy of primary root of alfalfa, 643
Animals, domestic, Corynebacteria associated with diseases of, 181
Anomodon attenuatus, water holding capacity of, 347
Aplanobacter stewartii, 477
Apanteles hartii Vier., 574
A. laeviceps Ash., 574
A. papapapam Mues., 573
Apples, storage behavior of, as influenced by nitrogen fertilization and storage temperature, 95
Applied botanical research on maize, 409, 411
Applied course in general botany, an, 299
Arearia interpres morinella, 610, 618
Arphia sulphurea Fab., 177
Aspergillus, five species of, growth indices for, 42, 43
A. flavus Link from alfalfa hay, 37
Attainments in teaching, 281
Avocet (Recurvirostra americana), 615
Bacteria, effect of, on ripening of cheddar cheese made from pasteurized milk, 173
Escherichia—Aerobacter group of, 209
Bacterial wilt of corn, dissemination of, 461
wilt of corn, relation of winter temperatures to distribution of, 477, 478
secondary infection of, by flea beetles, 467
wilt resistance in corn as caused by Bacterium stewartii, 539
Bacteriological studies on butter showing surface taint, 145
Bacterium stewartii (Smith) Migula, bacterial wilt of corn caused by, 539
Baird's sandpiper (Pisobia bairdi), 612, 620
Barbula unguiculata, on road banks, 350
Bartramia longicauda, 610
B. pomiformis, absorption of, 347
Bassus simillimus Cress., 575
Benecky, Charles Edwin, 216, 221, 227, 236, 259, 287
Biochemistry, of sluggish butyl-acetonic fermentations, the, 195
of the production of 2,3-butylene glycol by fermentation, the, 195
Biological and management aspects of game shooting, 636
Biology, and control of Neosciara ocelaris, 25
of Oncopeltus fasciatus (Dallas), 73
Black-bellied plover (Squatarola squatarola), 610, 618
Blood cell counts on cricket, method, 14
Black Mexican maize, chromosome studies in, 549
Bob-white, population, 626
winter survival on experimentally shot and unshot areas, 625
Botanical, research on maize, 411
science, commemorating modern era in, 221
science, evolution and differentiation of laboratory teaching in, 235
Botanists, challenge of erosion to, 353
Botany, applied course in general, 299
changing emphasis in, and significance, 287
content of general courses in, 253
notebooks, 291
place of, in liberal education, 243
placement test in teaching, 303
teaching general, 225
teaching, sixty-five years ago, 227
Brachythecium, artificial cultivation in erosion work, 350
Buff-breasted sandpiper (Tryngites subruficollis), 623
Butter, cultures, causes of slow acid production in, 135
surface taint of, as defect, 145
INDEX

Butyl-acetonic, fermentations, biochemistry of sluggish, 195
fermentation of the sugars with special reference to xylose, the, 197
Butyl organisms, action of, on carbohydrates, 197
2,3-Butylene glycol biochemistry of the production of, by fermentation, 171

Calidris canutus, 611
Caloptrophorus semiplanatus inornatus, 611
Capella delicata, 610, 619
Carbohydrates, in maize, translocation of, 509
Carbon dioxide, relation of temperature to production of in fungi, 52, 57
Carpocapsa pomonella Linne, toxicity of petroleum oil and chemical compounds to larvae of, 178
Catharinea, cultivation of, in erosion work, 350
Causes of slow acid production in butter cultures, 155
Ceratodon, for newly graded roadsides, 350
Certain aspects of the role of vegetation in erosion control, 337
Chaetocnema pulicaria, 468
Challenge of erosion to botanists, 353
Changing emphasis in general botany and its significance, 287
Charadrius melodus Ord., 609, 618
C. semipalmatus, 609, 618
Cheddar cheese, ripening of, Effect of bacteria on, 173
Chinch bugs, resistance and susceptibility of corn strain to second brood, 413
Chlorine sterilizing compounds, 163
Chortophaga viridifasciata, De G., 177
Chromosome, linkages, summary of 10th, 455
studies in Black Mexican maize I. Behavior of extra chromosomes in Black Mexican inbreds and hybrids with dent types of maize, 549
chromatic and meliotic, in Black Mexican maize lines and crosses, 550
Ciellus pygmaeus, new coccidium from, 581
Citrate of milk and products as they affect growth of Penicillium roqueforti, 151
Citrate of milk and products as they affect growth of Penicillium roqueforti, 151
Dissemination of bacterial wilt of, 461
dry weight, in planting rates, 525
future outlook for, 566
histological studies of, in study of bacterial wilt, 541
improvement, six decades of, and the future outlook, 561
inbred tests of, in relation to resistance to Bacterium stewartii, 539
increase in genetic knowledge of, 564
plants, leaf area and growth rate of, 521
production, future of, 559
production, problems relating to, 559
rate of planting, 523, 527
Reid Yellow Dent, spread of, 562
relation of field stands to yield, 529, 536
Research Institute, Iowa, 408, 411, 559
seed treatments, benefits of, 536
seed treatment, relation of rate of planting to the effect of, 527
smut, artificial inoculations with, 505
smut, place of attack, 505, 506
smut, unexpressed infection with, 507
stalks, basal area of, 523
strains, resistance and susceptibility of, to second brood chinch bugs, 413
yield of, effect of rates of planting on, 522
yield test, purpose of, 560

Constants, determination of, 599
Content of general botany courses, 253
Continuous loads, finite rectangle, 601
Commemorating six decades of the modern era in botanical science, 221
Condensation reactions of furfural and its derivatives, 141
Cornea, of guinea pig, 669
Cornstalks, production of synthetic lumber from, 183
Corynebacteria (Diptheroids) associated with diseases of domestic animals, 181
Culture tubes, metal jacketed glass, 590
Culture tubes, quartz and glass, 588
Dairy products, bacteria in, 209
Deflection, of an isotropic rectangular plate under the action of continuous and concentrated loads when supported at two opposite edges, the, 597
of a rectangular plate uniformly loaded, 607
Determination, of constants, 599
of the effect on manganese and sulfur on the malleabilization of white cast iron, 163
of the thermionic work function of nickel by a new method, the, 131
Development, of root and crown of alfalfa, 641
of the commercial production of refrigeration board and press board, 183
of the group-conference study of teaching, the, 259
Diabrotica duodecipunctata, summary of seed transmission tests with, 467
Diplodia zeae, effect of filtrates from cultures of, upon seedling blight of maize, 461
Dissemination of bacterial wilt of corn, 461
Dissoxteira carolina Linne, 177
Domestic animals, diseases of, Corynebacteria associated with, 161
Dowitcher, (Limnodromus griseus), 613, 622

Eastern solitary sandpiper (Tringa solitaria solitaria), 611, 619
Ectopimorpha luperinae Cush., 571
Effect of, inbreeding and of selection within inbred lines of maize upon the hybrids made after successive generations of selfing, the, 429
certain bacteria on the ripening of cheddar cheese made from pasteurized milk, the, 173
plant cover on soil and water losses, the, 329
species of grasses and legumes sown and treatment upon the population of meadows and pastures, 391
Eimeria beecheyi from ground squirrel, 581
E. beckeri n. sp., a new coccidium from the ground squirrel, Citellus pygmaeus, 581
E. miyairii, duration of infection of, 118
immunizing effects of different sized doses of, 119
infections in the white rat, 115
number of oocysts produced by different sized infective doses of, 116
weight changes, pathological effects and lethal dosage of, 121
Electrophoresis, 187
Elements, solubilities of, with vanadium oxytrichloride, 90
Epiblema strenuana (Walker), 576
Epiurus pterophori (Ashm.), 575
Equilibrium equations, 597
Ereunetes maurii, 613, 622
E. pusillus, 613, 622
Erosional losses, 399
Erosion, challenge of, to botanists, 353
control, certain aspects of role of vegetation in, 337
prevention, capacity of plant cover, 323
problems in relation to plant life, 326
regions presenting types of problems of, 357
Eroded soils, in southeastern Ohio, revegetation on, 365
Erosion, suggested plan for correcting damage from, 355
Erycia deckeri Curran, 269
Escherichia—Aerobacter group of bacteria in dairy products, 209
Ethylene chlorohydrin, use of, in germination tests on Jerusalem artichokes, 67
Euplomerinus viridescens (Walsh), 575
Evolution and differentiation of laboratory teaching in the botanical sciences, 235
Experiments on the attenuation of virus and the production of immunity to hog cholera, 192
Eye, defect, inherited in guinea pig, 667
tissue in guinea pig, 670
Fall migration of shore birds, Iowa, 617
Fermentation, bacterial count, 196
Fermentation of sugars, butyl-acetonic, 197
Fermentations, sluggish butyl-acetonic, biochemistry of, 195
thermophilic production of alcohols, by, 201
Field cricket (Gryllus assimilis pennsylvaniae Burm.), blood cell count of, 13
Filterability of hog cholera virus, as affected by hydrogen ion concentration, 187
Filters, compound, 190
gypsum, 189
Seitz-Uhlenhuth type of, 191
siliceous, 188
Filtrates from cultures of Diplodia zeae, 482
Forest and forest borders, revegetation of, 368
Forest and grass cover, effect on erosion losses, 334
Formalized tissues, 193
Fungi, isolation of, causing thermogenesis, 37
thermogenesis in hay-inhabiting, 37
Furan compounds, polymerization and stabilities of, 141
Furfural, and its derivatives, condensation reactions of, 141
use of polymers from, 133
Future of corn production, the, 559
Friedel-Crafts reaction, 142
Game management areas, 625
Gattermann-Koch reaction, 142
Genetic investigations of bacterial wilt resistance in corn as caused by Bac­terium stewartii (Smith) Migula, 539
Genetics, in relation to improvement of corn, 564, 565
Genetic investigations of bacterial wilt resistance in corn as caused by Bac­terium stewartii (Smith) Migula, 539
Grasses, effect of, and treatment upon population of meadows and pastures, 391
native, place in revegetation on eroded soils in Ohio, 370
population of mixed seedlines, 394
surviving, sown in pure seedlings, 393
Greater yellowlegs (Totanus melanoleucus), 611, 619
Ground squirrel, Eimeria beckeri, n. sp., from, 581
Group-conference system of teaching botany, 259
Growth rate of corn plants, 521
Gryllus assimilis pennsylvanicus Burm., total blood cell count, 13
Guinea pig, cornea, anatomical study of eye defect in, 667
Gymnochaeta ruficornis Will., 569
Helianthus tuberosus L., rest period of, 61
Heliothis zea Bodd., 45
Hemoglobin in the rat, 169
Hemolymp cell counts of Gryllus assimilis pennsylvanicus Burm., 16
Herpetomonas elmassiana (Migone), parasitic on Oncopeltus fasciatus, 84
Histological studies of the development of the root and crown of alfalfa, 641
Hog cholera, attenuation of virus, and production immunity to, 187, 192
Hog cholera, virus, migration of, when subjected to electrophoresis, 187
Hog cholera, virus, physical-chemical properties of, 187
Hormodendron, growth indices for species of, 45
Hudsonian godwit (Limosa haemastica), 614
Hydrolysis, acid, 206
Immunity to hog cholera, 192
Importance of outdoor plant studies, the, 325
Inbreeding, effects of, and of selection within inbred lines of maize upon the hybrids made after successive generations of selfing, 429
Insects, physiological and toxicological studies on, 177
Intracellular, abnormalities associated with yellow dwarf of onions, 677
bodies in onions, 678
Low Corn Research Institute, 409, 411
Isotropic rectangular plate, deflection of, 597
Insulation board, factors affecting production of, 183
Jerusalem artichokes, Helianthus tuberosus L., shortening the rest period of the tubers of the, 61
Kildeer (Oxyechus vociferus vociferus), 609, 618
Laboratory teaching, in botanical science, 235
Lactic acid bacteria, heterofermentative, 8
Lactobacillus casei, 173
Lactobacillus fructivorans sp. nov., physiology and classification of, 1
L. fructivorans, comparative studies on, 4
L. gracilis, comparative studies on, 4
Land use, planning of, 345
Leaf area and growth rate of corn plants, 521
Least sandpiper (Pisobia minutilla), 612, 620
Legumes, densities of surviving, sown in mixtures, 395
effect on, and treatment upon population of meadows and pastures, 391
Lesser yellowlegs (Totanus flavipes), 611, 620
Lignin, alkaline oxidation of, 205
Limnophorus griseus, 613, 622
Limosa fedra, 614
L. haemastica, 614
Line loads and concentrated loads, 602
Lišnóra brunnea (Cress.), 572
Liverworts, soil holding capacity, 349
Lixophaga variabilis (Coq.), 570
Lobipes lobatus, 615, 623
Loss mutations in maize, 427
Macrocenetus eliciatus Cress., 575
M. pallisteri Degrange, 575
Macronoicta onusta Grote, 576
Maize, acre yields of crosses of, 433, 434, 435
applied botanical research on, 411
Black Mexican, chromosome studies in, 549
Black Mexican, cytology of, 549
characters in top crosses of, 447
chromosome studies, 550
diurnal variations in rate of translocation in, 514
effect of filtrates from cultures of Diplodia zeae upon seedling blight of, 481
effect of selection between sister progenies, 431
formative period of stem structures in, 493
inbred lines of, effect of self pollination on, 429
loss mutations in, 427
morphological relationships of stem and root of, 489
plant, ontogeny of, 489
relation of fruiting to translocation in, 509
relation of leaf area to yield of, 510
smt, some new aspects of, 505
some new mutants in, 451
sugar gradients in, 515
translocation of carbohydrates in, 509
vascular bundles, relationship between size and number, 494
Manganese and sulfur, effect of, on malleability of white cast iron, 165
Marbled godwit (Limosa fedoa), 614
Masicera senilis Meig., 567
Medicago sativa, anatomical development of, 641
embryo of, 653
histological studies of, 642
INDEX 691

P. melanotos, 611, 620
P. minutilla, 611, 620
Place of Botany in a liberal education, the, 243
Placement test, use of, in teaching botany, 303
Plant cover, density, run-off as measured by, 399, 403
effect of, on soil and water losses, 329
percentage of, in successional development of, 380
Plant covers, effectiveness of, in preventing run-off losses, 407
Plant studies, importance of outdoor, 325
Plant succession, relation of, to soil erosion, 379
Plastics from high pentosancellulosic material, 159
Plastic material, standard process for producing, 159
Pluvialis dominica dominica, 610, 618
Pohlia nutans, slopes helped by, 348
Polymerization and stabilities of furan compounds, 141
Polymers, use of, from furfural in the fabrication of molded products, 133
Popping seed in Mexico used as substitute for popcorn, 661
Population check of bob-white on shot and unshot areas, 634
Potato odor in milk, organisms producing, 125
Preliminary, report of the anatomical study of an inherited eye defect in the guinea pig, 667
studies on the effect of filtrates from cultures of Diplodia zeae upon seedling blight of maize, 481
Pressboard, commercial production of, 183
Primary root of alfalfa, anatomy of, 643
Production of alcohols by thermophilic fermentations, the, 201
Products of dissimilation of glucose and levulose, 5
Pseudomonas graveolens, ability to cause odor defects in dairy products, 125
P. mucidolens, changes in flavor of cream caused by, 126
Quantitative studies on the formation of xylose from pentosan-containing materials, 137
Quail, control of shooting for sport, 639
population surviving shooting, 638
Rat, growth and lactation in the, 147
role of inorganic substance and amino acids in regeneration of hemoglobin in the, 169
Rate of planting of corn, 523
Ratio of sugar to nitrogen in apples, 105
Recurvirostra americana, 615
Red-backed sandpiper (Pelidna alpina sakhalina), 612, 622
Refrigeration board, commercial production of, 183
Relation, of rate of planting to the effect of corn seed treatment, 527
of the size of the infective dose to number of oocysts eliminated, duration of infection, and immunity in Eimeria miyairii Ohira infections in the white rat, 115
of the stages of plant succession to soil erosion, the, 379
Research, on maize, 411
Resistance and susceptibility of corn strains to second brood chinch bugs, 413
Revegetation on eroded soils in southeastern Ohio, 365
Role of inorganic substances and amino acids in the regeneration of hemoglobin in the rat, 169
Ruddy Turnstone (Arenaria interpres morinella), 610, 618
Run-off measurement of, as influenced by plant cover density, 399
Sagaritis oxylus (Cress.), 572
Salad dressing, fermented, Lactobacillus fructivorans isolated from, 1
Sanderling (Croethia alba), 614, 623
Sarcophaga cimbicis Towns., 571
S. helicis Towns., 570
Second brood chinch bugs, resistance and susceptibility of corn strains to, 413
Secondary growth of alfalfa, changes in, 647
Seed treatment, corn, 527
Semipalmated plover (Charadrius semipalmatus), 609, 618
Semipalmated sandpiper (Ereunets pusillus), 613, 622
Shore birds, migration of, in Iowa, 609
Shortening the rest period of the tubers of the Jerusalem artichoke, Helianthus tuberosus L., 61
Sinea diadema (Fab.), as predator, 84
Six decades of corn improvement and the future outlook, 561
Slopes, held by mosses, 348
Smut, maize, 505
Sodium nitrate fertilizer applications in orchards, 111
Sodium thiocyanate, effectiveness of in shortening rest period of tubers, 71
Soil, and water-losses under crop rotations, 335
characteristics, in stages of plant succession, 385
erosion, mosses and, 347
relation of stages of plant succession to, 379
formation, rate of, 329
water-holding capacity of, 381
Solvent yield, influence of factors on, 198
Some factors affecting the growth of Penicillium roqueforti in cheese, 151
Some factors affecting the production of insulation board on the development of the commercial production of refrigeration board and pressboard, 183
Some aspects of maize smut, 505
Some new mutants in maize, 451
Some of the physical-chemical properties of hog cholera virus
I. Filterability of hog cholera virus as affected by the hydrogen ion concentration
II. The migration of hog cholera virus where subjected to electrophoresis
III. Experiments with the attenuation of virus and the production of immunity to hog cholera, 187
Some problems in an elementary botany course, 249
Some quantitative studies of organometallic compounds, 213
Spicaria, growth indices for species of, 45
Sporobolus wrightii, purpose of, in Southwest, 354
Spotted sandpiper (Actitis macularia), 610, 619
Spring migration of shore birds through Clay and Palo Alto counties, Iowa, the 1934, 609
Squatarola squatarola, 610, 618
Stages of plant succession, relation of, to soil erosion, 379
Stegapopous tricolor, 615, 623
Stem and root structure of maize, 489
Storage, behavior of apples as influenced by nitrogen fertilization and storage temperature, the, 95
length and maturity, effect of on germination of artichoke tubers, 63
temperature of apples, 95
Streptococcus lactis, 153
S. liquefaciens, 173
S. paracitrovorus, 173
Stilt sandpiper (Microptila himantopus), 613, 622
Studies on the Escherichia-Aerobacter group of bacteria in dairy products, 209
Studies on the vitamins B and G in growth and lactation in the rat
(a) The effects of extracts of vitamin B and G
(b) The distribution of vitamin G, 147
Study of chlorine sterilizing compounds, I. Relationship between pH and oxidation potentials, 163
Study of Corynebacteria (Diphtheroids) associated with diseases of domestic animals, A, 181
Sugars, butyl-acetonic fermentation of, 197
Sugar content of apples, 102
Susceptibility of corn strains to second brood chinch bugs, resistance and, 413
Symposia of, Applied botanical research on maize, 409
Erosion prevention capacity of plant cover, 323
Teaching general botany, 219
Teaching, attainments in, 281
general botany, 225
symposium, 219
group-conference, for botany, 259
of botany sixty-five years ago, 227
Thermionic work function of nickel by a new method, determination of the, 131
Thermogenesis, 47
determination of, 40
in hay-inhabiting fungi, 37
Thermophilic fermentations, production of alcohols by, 201
Thiourea, use of, in germination test on Jerusalem artichoke tubers, 67
Tissues, desiccated, 192
Totanus flavipes, 611, 620
T. melanoleucus, 611, 619
Toxicity of petroleum oil mixed with certain chemical compounds to larvae of Carpocapsa pomonella Linne, 178
Toxicoological studies on insects, 177
Trachaea, in roots of maize plants, cross sectional area, 494, 497
Translocation of carbohydrates in maize, the, 509
Tringa solitaria solitaria, 611, 619
Tryngites subfriculola, 623
Upland plover (Bartramia longicauda), 610
Use of polymers from furfural in the fabrication of molded products, 133
Use of the placement test in the teaching of general botany, a, 303
Vanadium oxytrichloride, as a solvent, II, 159
physical properties of, 89
preparation of, 89
reactions with sulfur dioxide, hydrogen sulfide and Grignard reagent, 93
relationships between the elements and, 89
Vascular bundles of maize, at internodes, 492
Vascular transition from root to crown of alfalfa, 645
Vectors of virus diseases of onions, 677
Vegetative communities in eroded areas, in Ohio, 366
Vegetation, role of, in erosion control, 337
Virus, attenuation of, 192
hog cholera, physical-chemical properties of, 187
saturation of, with gases, 193
Vitamin G, distribution of, 147
Vitamins B and G, extracts of, 147
in growth and lactation in the rat, 147
Water-holding capacity of soil, 381
Water, losses, effect of plant cover on soil and, 329
municipal and irrigation use, 345
Western sandpiper (*Ereunetes maurii*), 613, 622
Western willet (*Caloptrophorus semi-palmatus inornatus*), 611
White-rumped sandpiper (*Pisobia fusci-collis*), 611, 620
Wilson's phalarope (*Steganopus tricolor*), 615, 623
Wilson's snipe (*Capella delicata*), 610, 619
Winter survival, bob-white, on shot and unshot areas, 625

*Winthemia rufopicta* (Bigot), 569
Xerosere, successive stages of, dominant species of, 382, 384
Xylose, butyl-acetonic fermentation of sugars with special reference to, 197
formation of, from pentosan-containing materials, 137
Yeast, cultures, effect of metals on, 587
mitogenetic effect on, of oligodynamic radiation from metals, 587
Yellow dwarf of onions, abnormalities associated with, 677