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# HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES. II. SULFIDES, SULFOXIDES, AND SULFONES[1]

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During the course of investigations in these laboratories concerned with the utilization of animal by-products, a number of long-chained aliphatic sulfur compounds have been prepared. These include the even-chained *n*-alkyl sulfides from dodecyl to octadecyl and the corresponding sulfoxides and sulfones. The sulfides were prepared by the action of alcoholic sodium sulfide on the corresponding alkyl bromides. The method is remarkably simple, and it is surprising that none of the symmetrical *n*-alkyl sulfides above octyl have been described. A U. S. patent[2] refers to the production of aliphatic sulfides by the interaction of an inorganic sulfide and straight-chained alkyl halides of ten to fifteen carbon atoms. Evidently the sulfides between decyl and pentadecyl have been prepared under this patent, but their description appears to have been withheld. No reference is given to the corresponding sulfoxides and sulfones. Cetyl sulfide was prepared by Fridau[3] in 1852, but the sulfoxide and sulfone were not described. v. Pieverling[4] attempted to prepare mellisyl ($C_{31}$) sulfide but obtained the mercaptan instead. Jones and Reid[5] have prepared a number of long-chained, unsymmetrically substituted sulfides by the addition of mercaptans to unsaturated hydrocarbons and have proved their structure by synthesis from the potassium salt of mercaptans and the normal alkyl halides.

A great variety of oxidizing agents has been used to convert sulfides to the corresponding sulfoxides and sulfones. Grabowsky[6] oxidized butyl sulfide to the sulfoxide with dilute nitric acid. When fuming nitric acid was used, butyl sulfone was the product.

Hinsberg[7] treated a number of sulfides and disulfides with the calculated quantities of hydrogen peroxide in acetic acid and was able to obtain the corresponding sulfoxides and disulfoxides. Hydrogen peroxide in acetone was used by Gazdar and Smiles[8] to produce sulfoxides. These investigators concurred with Hinsberg in the observation that the oxidation of sulfides with hydrogen peroxide may be readily controlled at ordinary temperature so as to proceed only to the sulfoxide stage. Excess peroxide at elevated temperature yields sulfones.

[1] The first paper in this series is: Gilman and Ford, *Iowa State College J. Sci.* 13, 135, (1939).
[2] U. S. patent, 2,085,452, [*C. A.*, 31, 5812 (1937)].
[3] Fridau, *Ann.*, 83, 16 (1852).
[4] v. Pieverling, *Ann.*, 183, 349 (1876).
[5] Jones and Reid, *J. Am. Chem. Soc.*, 60, 2452 (1938).
[6] Grabowsky, *Ann.*, 175, 348 (1875).
[7] Hinsberg, *Ber.*, 41, 2836 (1908).
[8] Gazdar and Smiles, *J. Chem. Soc.*, 93, 1834 (1908).

Chromic acid in acetic acid solution was declared by Knoll[9] to be an excellent reagent for the oxidation of sulfides to sulfoxides. Our experience has shown that in the presence of a considerable excess of chromic acid, sulfones may also be produced.

Fries and Vogt[10] have used moist chlorine or bromine to form sulfoxides. The intermediate thio ether dichloride or dibromide is first formed and this then undergoes hydrolysis to the sulfoxide.

Bost and co-workers[11] prepared long-chained alkyl 2,4-dinitrophenyl thio ethers and oxidized these to sulfones, using potassium permanganate in acid solution.

Sodium hypochlorite solution has been used by Woods and Travis[12] in the preparation of aliphatic and aromatic sulfones. According to these workers, this reagent seems to be best adapted to the oxidation of alkyl sulfides below heptyl.

Benzoyl peroxide appears to be a suitable oxidizing agent for the quantitative formation of both aromatic and aliphatic sulfones.[13]

Fichter and Sjöstedt[14] have used electrolytic oxidation to convert certain sulfides to sulfoxides and sulfones.

We have chosen dilute nitric acid as the most suitable reagent for oxidation of the long-chained alkyl sulfides to sulfoxides. For the formation of the corresponding sulfones, either an acetic acid solution of hydrogen peroxide or fuming nitric acid are to be recommended, the former reagent probably being more convenient.

The insecticidal properties of the sulfides and their oxidation products are being investigated.

The melting points and yields of the sulfides, sulfoxides, and sulfones discussed in this work are given in Table I.

TABLE I

MELTING POINTS AND YIELDS OF SULFIDES, SULFOXIDES, AND SULFONES

| | SULFIDES | | SULFOXIDES | | SULFONES | |
|---|---|---|---|---|---|---|
| | m. p. | Yield % | m. p. | Yield % | m. p. | Yield % |
| *n*-Dodecyl ..... | 40-40.5 | 81 | 89-90 | 95 | 94.5-95.5 | 95 |
| *n*-Tetradecyl ... | 49-50 | 70 | 95-96 | 35 | 99.5-100 | 90 |
| *n*-Hexadecyl .... | 57-58 | 76 | 97-98 | 50 | 100-100.5 | 85 |
| *n*-Octadecyl .... | 68-69 | 75 | 99-100 | 92 | 105.5-106.5 | 90 |

The yields herein reported are not necessarily maximum yields. This is particularly true in the case of *n*-tetradecyl and *n*-hexadecyl sulfoxides where difficulty was encountered in purifying the substances.

[9] Knoll, *J. prakt. Chem.*, **113**, 40 (1926).
[10] Fries and Vogt, *Ann.*, **381**, 338 (1911).
[11] Bost, Turner and Norton, *J. Am. Chem. Soc.*, **54**, 1986 (1932).
[12] Woods and Travis, *J. Am. Chem. Soc.*, **50**, 1226 (1928).
[13] Lewin, *J. prakt. Chem.*, **118**, 282 (1928).
[14] Fichter and Sjöstedt, *Ber.*, **43**, 3422 (1910).

## EXPERIMENTAL PART

### PREPARATION OF ETHYL STEARATE[15]

Stearic acid (250 g.) was dissolved in 300 cc. of warm absolute ethyl alcohol, and the resulting solution completely saturated with dry hydrogen chloride. After standing at room temperature for six hours the entire mixture was poured into warm water, and the ester layer separated and washed several times with fresh portions of warm water. The product was taken up in 100 cc. of ether and dried over sodium sulfate. After removal of the ether, the ester distilled very constantly at 190-192° (4 mm.). Yield: 248 g. (90 per cent).

### PREPARATION OF *n*-OCTADECANOL[16]

Sixty-five grams (0.2 mole) of ethyl stearate was placed in a 500 cc. bomb of a Parr hydrogenation machine. Six grams of copper-chromium oxides catalyst[17] was added and the bomb filled with hydrogen (1,640 lbs. pressure at 25° C.). The temperature was raised to 250° and maintained there for four and one-half hours. After cooling, the product was removed, dissolved in hot acetone, and filtered. Fifty-two grams (94 per cent) of *n*-octadecanol (m. p. 57-58°) crytallized from the cooled filtrate.

### PREPARATION OF *n*-OCTADECYL BROMIDE[18]

A mixture of 38 g. of 42 per cent hydrogen bromide and 13 g. of concentrated sulfuric acid was placed in a 250 ml. 3-necked flask equipped with a mechanical stirrer and reflux condenser. Twenty-seven grams (0.1 mole) of *n*-octadecanol was gradually added and the reactants refluxed with stirring for six hours. Another addition of 19 g. of 42 per cent hydrogen bromide was made and the refluxing continued for one hour. The reaction mixture was poured into water, the bromide layer separated, washed with cold concentrated sulfuric acid, then with water, and finally with sodium carbonate solution. The oil was taken up with ether, and washed once more and the ether solution dried over sodium sulfate. After removal of the ether (water bath) the bromide distilled at 194-195° (6 mm.). Yield: 30.5 g. (91 per cent). A variation of this procedure has been used by McCorkle.[19]

### PREPARATION OF *n*-OCTADECYL SULFIDE

Five grams (0.22 gram atom) of sodium metal was dissolved in 200 cc. of absolute ethyl alcohol. The solution was divided into two equal portions; the first part was saturated with dry hydrogen sulfide and to this was added the second part of the sodium ethylate solution. A solution of 26.8 g. (0.08 mole) of *n*-octadecyl bromide in 200 cc. of absolute

[15] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 89. (Unpublished).
[16] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 90. (Unpublished).
[17] Folkers and Connors, *J. Am. Chem. Soc.*, 54, 1138 (1932).
[18] Hoyt, Doctoral thesis No. 560, Library, Iowa State College (1940). p. 96.
[19] McCorkle, Doctoral thesis, No. 481, Library, Iowa State College, 1938, p. 85.

alcohol was then added to the alcoholic sodium sulfide solution. The mixture was refluxed overnight (eight hours) on a steam bath and was then filtered hot. Upon cooling the filtrate, the sulfide crystallized in the form of lustrous plates. These were filtered off and melted in distilled water. After cooling, the solid was removed and melted as before until the water no longer showed any cloudiness in the presence of the melted sulfide. The product was allowed to dry and when recrystallized from acetone weighed 16 g. (75 per cent) and melted at 68-69°.

*Anal.* Calcd. for $C_{36}H_{74}S$: S, 5.94 per cent. Found: S, 5.93 per cent.

PREPARATION OF *n*-OCTADECYL SULFOXIDE

(A) One gram (0.00185 mole) of *n*-octadecyl sulfide was dissolved in 100 cc. of hot glacial acetic acid. To this was added 0.25 g. (0.0025 mole) of chromic acid ($CrO_3$) in ½ cc. of water and 5 cc. of acetic acid. The brownish-green solution that immediately developed changed to a clear green solution. The mixture was kept warm for two hours, cooled, and the crystals filtered off. Upon recrystallization from acetic acid, these melted at 99-100° and weighed 0.92 g. (92 per cent).

*Anal.* Calcd. for $C_{36}H_{74}SO$: S, 5.77 per cent. Found: S, 5.86 per cent, 5.65 per cent.

(B) One gram of *n*-octadecyl sulfide was added to 100 cc. of dilute nitric acid (50 cc. concentrated nitric acid in 50 cc. of water). The mixture was warmed on a steam bath for thirty minutes, cooled, filtered, and the solid material recrystallized from acetone. The product weighed 0.3 g. (30 per cent) and melted at 99-100°. A mixed melting point with the product of (A) above showed no depression.

PREPARATION OF *n*-OCTADECYL SULFONE

(A) One gram of *n*-octadecyl sulfide was dissolved in 100 cc. of warm glacial acetic acid. Five cubic centimeters of 30 per cent hydrogen peroxide was added and the solution kept warm for four hours. The solution was cooled and the crystals filtered off. The dried product weighed 0.9 g. (90 per cent) and melted at 105.5-106.5°.

*Anal.* Calcd. for $C_{36}H_{74}SO_2$: S, 5.61 per cent. Found: S, 5.61 per cent, 5.58 per cent.

(B) One gram of *n*-octadecyl sulfide was added to 50 cc. of fuming nitric acid and the mixture warmed on a steam bath for one hour. The acid mixture was poured into water, filtered, and the solid recrystallized from acetic acid. One-half gram of product (50 per cent yield) was obtained which melted at 105.5-106.5°. A mixed melting point with the sulfone obtained in (A) above was not depressed.

(C) In an effort to make *n*-octadecyl sulfoxide from 1 g. of the sulfide and 0.25 g. of chromic acid in acetic acid, a product was obtained from one run that melted low (92-96°). This was treated once more with 0.25 g. of chromic acid in acetic acid. The product weighed one gram and melted sharply at 105.5-106.5°. A mixed melting point with an authentic specimen of *n*-octadecyl sulfone showed no depression. This is significant

inasmuch as Knoll[20] states that even though a moderate excess of chromic acid is used the sulfoxide will be the product.

REDUCTION OF *n*-OCTADECYL SULFOXIDE TO *n*-OCTADECYL SULFIDE

One-half gram of *n*-octadecyl sulfoxide was dissolved in 100 cc. of hot acetic acid. Zinc dust (2 g.) was added and the mixture refluxed for ten hours. The solution was filtered and allowed to cool. Silvery plates deposited which melted over a wide range (68-85°). The material was redissolved in acetic acid and refluxed with zinc dust for ten hours more. The hot solution was filtered, cooled, the crystals filtered off and washed with water. The dried product melted at 68-70°, and when mixed with pure *n*-octadecyl sulfide, the melting point was 68-70°. The yield was practically quantitative.

OXIDATION OF *n*-OCTADECYL SULFOXIDE TO *n*-OCTADECYL SULFONE

One-half gram of *n*-octadecyl sulfoxide was dissolved in 100 cc. of acetic acid and 3 cc. of 30 per cent hydrogen peroxide added. The mixture was warmed for two hours, allowed to cool, and the crystals filtered off. After thorough washing and drying, the sulfone was obtained in quantitative yield and melted at 105.5-106.5°. A mixed melting point with pure *n*-octadecyl sulfone showed no depression.

ATTEMPTED REDUCTION OF *n*-OCTADECYL SULFONE

One-half gram of *n*-octadecyl sulfone was dissolved in 80 cc. of hot acetic acid. To this was added 3 g. of zinc dust, and the mixture was refluxed for ten hours. The hot solution was filtered, cooled and the product filtered off. After washing and drying, the sulfone was quantitatively recovered and melted at 105.5-106.5°. A mixed melting point determination with pure *n*-octadecyl sulfone showed no depression. These results are in agreement with the generally known fact that sulfones are not reduced by the action of zinc in acids, whereas sulfoxides are reduced to sulfides by this treatment.

PREPARATION OF *n*-HEXADECYL BROMIDE

*n*-Hexadecyl bromide was prepared by the same procedure as that used for *n*-octadecyl bromide. Forty-eight and four-tenths grams (0.2 mole) of *n*-hexadecanol yielded 44 g. (72 per cent) of *n*-hexadecyl bromide boiling at 178-179° (5 mm.).

PREPARATION OF *n*-HEXADECYL SULFIDE

In accordance with the procedure as given under *n*-octadecyl sulfide, 20 g. (0.065 mole) of *n*-hexadecyl bromide yielded 12 g. (76 per cent) of *n*-hexadecyl sulfide melting at 57-58°.

*Anal.* Calcd. for $C_{32}H_{66}S$: S, 6.64 per cent. Found: S, 6.44 per cent.

PREPARATION OF *n*-HEXADECYL SULFOXIDE

Following the method described for *n*-octadecyl sulfoxide, 1 g. of *n*-hexadecyl sulfide upon oxidation in dilute nitric acid gave 0.5 g. (50 per cent) of *n*-hexadecyl sulfoxide. Some difficulty was encountered

[20] Knoll, *J. prakt. Chem.*, **113**, 40 (1926).

when the product was being purified by melting in water. An emulsion was formed that was broken only upon addition of acid. The substance so obtained was filtered off and recrystallized from acetone, m. p. 97-98°.

*Anal.* Calcd. for $C_{32}H_{66}SO$: S, 6.42 per cent. Found: 6.28 per cent.

PREPARATION OF *n*-HEXADECYL SULFONE

*n*-Hexadecyl sulfone was prepared by oxidation of 1 g. of *n*-hexadecyl sulfide with 5 cc. of 30 per cent hydrogen peroxide in 100 cc. of glacial acetic acid. The product obtained in 85 per cent yield (0.85 g.) melted at 100-100.5° after recrystallization from acetic acid.

*Anal.* Calcd. for $C_{32}H_{66}SO_2$: S, 6.23 per cent. Found: S, 6.21 per cent.

PREPARATION OF *n*-TETRADECYL BROMIDE

*n*-Tetradecyl bromide was prepared in the same manner as *n*-octadecyl bromide. One hundred and fifteen grams (0.54 mole) of *n*-tetradecanol gave 111 g. (75 per cent) of *n*-tetradecyl bromide. This substance boiled at 153-154° (6 mm.).

PREPARATION OF *n*-TETRADECYL SULFIDE

Following the same procedure as given for *n*-octadecyl sulfide, 20 g. (0.072 mole) of *n*-tetradecyl bromide yielded 11 g. (70 per cent) of *n*-tetradecyl sulfide. The product melted at 49-50° after recrystallizing from acetone.

*Anal.* Calcd. for $C_{28}H_{58}S$: S, 7.51 per cent. Found: S, 7.70 per cent.

PREPARATION OF *n*-TETRADECYL SULFOXIDE

One gram of *n*-tetradecyl sulfide was oxidized with dilute nitric acid in the same manner as described under *n*-octadecyl sulfoxide. Similar difficulty was encountered as with the purification of *n*-hexadecyl sulfoxide, and only 0.35 g. (35 per cent) of *n*-tetradecyl sulfoxide was recovered. The substance after recrystallization from acetone melted at 95-96°.

*Anal.* Calcd. for $C_{28}H_{58}SO$: S, 7.25 per cent. Found: S, 7.20 per cent.

PREPARATION OF *n*-TETRADECYL SULFONE

*n*-Tetradecyl sulfide (1 g.) was oxidized to *n*-tetradecyl sulfone by 5 cc. of 30 per cent hydrogen peroxide in 100 cc. of acetic acid. Nine-tenths gram (90 per cent) of the sulfone was obtained after recrystallization from acetic acid and melted at 99.5-100°.

*Anal.* Calcd. for $C_{28}H_{58}SO_2$: S, 6.98 per cent. Found: S, 6.97 per cent.

PREPARATION OF *n*-DODECYL BROMIDE

The preparation of *n*-dodecyl bromide was similar to that of *n*-octadecyl bromide. It was found advantageous, however, to add a small amount of dilute sulfuric acid to the water used in washing the ether solution of the bromide. The ether solution was dried with sodium sulfate and the solvent removed on a steam bath. The residual oil distilled between 135° and 145° (3 mm.). This was redistilled, and from the 57 g.

(0.3 mole) of *n*-dodecanol used, 70 g. (90 per cent) of *n*-dodecyl bromide was obtained boiling at 175-178° (45 mm.).

A similar preparation of *n*-dodecyl bromide has been given by Kamm and Marvel.[21]

### PREPARATION OF *n*-DODECYL SULFIDE

Preparation of *n*-dodecyl sulfide was similar to that of *n*-octadecyl sulfide. Twenty grams (0.08 mole) of *n*-dodecyl bromide yielded 12 g. (81 per cent) of *n*-dodecyl sulfide. This product was recrystallized from acetone and melted at 40-40.5°.

*Anal.* Calcd. for $C_{24}H_{50}S$: S, 8.65 per cent. Found: S, 8.66 per cent.

### PREPARATION OF *n*-DODECYL SULFOXIDE

One gram of *n*-dodecyl sulfide was oxidized with dilute nitric acid in the same manner as was *n*-octadecyl sulfide. The product when recrystallized from acetone melted at 89-90° and weighed 0.95 g. (95 per cent).

*Anal.* Calcd. for $C_{24}H_{50}SO$: S, 8.28 per cent. Found: S, 8.27 per cent.

### PREPARATION OF *n*-DODECYL SULFONE

One gram of *n*-dodecyl sulfide was oxidized in acetic acid solution with 5 cc. of 30 per cent hydrogen peroxide. The product weighed 0.95 g. (95 per cent) and after recrystallization from acetic acid melted at 94.5-95.5°.

*Anal.* Calcd. for $C_{24}H_{50}SO_2$: S, 7.95 per cent. Found: S, 7.76 per cent.

*Acknowledgements:* The author is grateful to Professor Henry Gilman for guidance, and to Dr. A. W. Ralston for helpful suggestions as well as for liberal supplies of some of the initial materials.

## SUMMARY

A number of long-chained *n*-alkyl sulfides, sulfoxides, and sulfones have been prepared.

[21] Gilman, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, New York (1932), p. 27.

# HIGH MOLECULAR WEIGHT FATTY ACID DERIVATIVES. III. CARBOXYLIC ACID SALTS AND AMIDES OF *n*-DODECYLAMINE AND *n*-OCTADECYLAMINE[1]

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The generally used methods for the formation of substituted amides are indirect in the sense that the amine does not react directly with an acid itself, but rather with a derivative of an acid such as an acid halide, an acid anhydride, or an ester. Conversion of the acid into these derivatives is time-consuming, and the yields are not always satisfactory. It seems that the more direct method of forming the amine salt of the carboxylic acid and subsequent dehydration of this to the amide is a procedure that has been overlooked to a great extent and probably can be applied advantageously in the preparation of a large number of substituted amides. The method is not a new one, but current references to its application appear to be limited almost entirely to the patent literature.

The first preparation of a substituted amide by pyrolysis of a substituted ammonium salt appears to be that of Wurtz[2] in 1850. Following the method used twenty years earlier by Dumas[3] for the conversion of ammonium oxalate to oxamide, Wurtz distilled dimethylammonium oxalate and obtained dimethyl oxamide. The method was extended to monobasic aliphatic acids by Linneman[4] who evaporated aqueous solutions of methyl-, ethyl- and diethylammonium formates and distilled the resulting syrups to obtain the corresponding amides. The isolation of the intermediate ammonium salt was eliminated by Franchimont and Klobbie[5] who heated *n*-heptylic acid and several low-molecular-weight amines directly in sealed tubes at about the boiling point of the acid and obtained the desired amides. Tafel and Stern[6] carried the direct condensation technique over to the field of dibasic acids. By heating isopropylamine and succinic acid in a sealed tube they were able to obtain isopropyl succinimide in 75 per cent yield.

Decomposition of acetic acid salts of a number of lower primary and secondary amines was quantitatively studied by Musselius.[7] The salts were heated in sealed tubes at 215° (in nitrobenzene vapor) for thirty minutes, and the unchanged salt was determined by titration with base.

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[1] Paper II: *Iowa State Coll. J. of Sci.*, 15, 215 (1941).

[2] Wurtz, *Ann. chim.*, 30, 464 (1850).

[3] Dumas, *Ann. chim. phys.*, 44, 129 (1830).

[4] Linneman, *Proc. Viennese Academy*, 60, 44 (1870); [*Chem. Zentr.*, 41, 138 (1870)].

[5] Franchimont and Klobbie, *Rec. trav. chim.*, 6, 247 (1887).

[6] Tafel and Stern, *Ber.*, 33, 2232 (1900).

[7] Musselius, *J. Russ. Phys. Chem. Soc.*, 32, 29 (1900). [*Chem. Zentr.*, 71, I, 1071 (1900)].

He found that the yield of amide increased with the molecular weight of the amine.

Verley[8] prepared dimethyl formamide and dimethyl acetamide by distilling an alkali salt of the acid and dimethylamine hydrochloride. Reid and co-workers[9] prepared the dimethyl amides of the normal fatty acids from formic to heptoic by passing dimethylamine through the acids at temperatures ranging from 95° to 160° for varying lengths of time, the conditions depending upon the acid used. Phosphorus pentoxide has been used as a dehydrating agent in the formation of certain N-substituted amides.[10]

Several propionamides have been prepared by Bowen and Smith[11] by refluxing amines with propionic acid for several hours. A U. S. patent[12] refers to the production of alkyl substituted amides by heating aliphatic acids and aliphatic amines in the presence of a liquid immiscible with water. The constant boiling mixture is distilled off, removing the water formed in the reaction.

A number of amides from carboxylic acids and *n*-dodecylamine and *n*-octadecylamine has been prepared. Harber[13] described the *o*- and *p*-chlorobenzamides, *o*- and *m*-toluamides, oleamides, elaidamides and anisamides of these amines. N,N'-Di-*n*-octadecyl malonamide and N-*n*-dodecyl and N-*n*-octadecyl phthalimide were also described by the same investigator. A number of the amides reported in Tables I and II have also recently been prepared by Harber [13] who used a modification of the method described in the Experimental Part. The modification consisted of eliminating the step involving the isolation of the intermediate salts. Mixed melting point determinations indicated that the products of either method were identical. N-*n*-Dodecyl lauramide has been prepared from a mixture of lauramide and lauric acid by heating with hydrogen at elevated temperature and pressure in the presence of a catalyst.[14] Adam and Dyer[15] prepared N-*n*-octadecyl acetamide and N-*n*-hexadecyl acetamide by warming the corresponding amines with acetic anhydride. N-*n*-Dodecyl acetamide is described in the patent literature as having been prepared by heating lauronitrile and acetamide with hydrogen under pressure in the presence of nickel.[16] N-*n*-Dodecyl benzamide, N-*n*-dodecyl phenylacetamide and N,N'-di-*n*-dodecyl oxamide were prepared by Grünfeld[17] from *n*-dodecylamine and the corresponding ethyl esters. N-*n*-Octadecylbenzamide has been previously prepared by the action of benzoyl

[8] Verley, *Bul. soc. chim.*, [3] 9, 691 (1893).

[9] Mitchell and Reid, *J. Am. Chem. Soc.*, 53, 1879 (1931); Ruhoff and Reid, *ibid.*, 59, 401 (1937).

[10] German Patent 653,873 [*C. A.*, 32, 2956 (1938)]

[11] Bowen and Smith, *J. Am. Chem. Soc.*, 62, 3522 (1940).

[12] U. S. Patent 1,954,433 [*C. A.*, 28, 3741 (1931)].

[13] Harber. Doctoral Thesis, No. 590, Iowa State College (1940).

[14] German Patent 667,627 [*C. A.*, 33, 2906 (1939)].

[15] Adam and Dyer, *J. Chem. Soc.*, 127, 73 (1925).

[16] British Patent 458,454 [*C. A.*, 31, 3501 (1937)]; German Patent 650,664 [*ibid.*, 32, 953 (1938)].

[17] Grünfeld, *Ann. chim.*, [10] 20, 366 (1933).

chloride on *n*-octadecylamine.[18, 20] Hoyt [19] prepared N-*n*-octadecyl stearamide from stearyl chloride and *n*-octadecylamine. Chloroacetic amides of *n*-dodecyl-, *n*-tetradecyl- and *n*-octadecylamine are mentioned in the patent literature,[21] but the physical constants and methods of preparation are not given.

A number of amides from long-chained amines other than *n*-dodecylamine and *n*-octadecylamine have been reported. Turpin[22] prepared N-*n*-heptadecyl benzamide from *n*-heptadecylamine and benzoyl chloride. Jeffreys[23] used the same method to form N-*n*-pentadecyl benzamide from *n*-pentadecylamine. The acetyl derivatives of these same amines as well as that of *n*-undecylamine have been described by Naegeli and coworkers.[24] These latter investigators also reported the formation of N-*n*-heptadecyl stearamide and N-*n*-pentadecyl palmitamide.

A number of substituted amides from long-chained acids and various amines are reported in the literature. de'Conno[25] mixed equimolar amounts of higher fatty acids and aromatic amines and heated these in evacuated, sealed tubes at 230° for five hours. The yields of amides were good. The *p*-nitroanilides and 2-nitro-*p*-toluidides of lauric, myristic, palmitic and stearic acids were prepared by Gilman and Ford[26] by heating the amines with the corresponding acid chlorides. The *p*-xenylamides of the same acids were prepared by these workers by heating equimolar quantities of the acids and *p*-xenylamine in sealed tubes for five hours at 135-140°. Robertson [27] prepared the *o*- and *p*-toluidides, β-naphthylamides, *p*-bromoanilides, *o*-bromo-*p*-toluidides, α-bromo-β-naphthylamides, and 2,4,6-tribromoanilides of a large number of normal fatty acids and made an interesting study of their melting points. He plotted the melting points of each series of compounds against the number of carbons in the acid radical and noted that the odd and even members fell on two distinct curves, which, although not superimposable, fall and rise in the same manner at corresponding places. A minimum in the curves appeared generally at about $C_8$. We find that similar results are obtained when one plots the melting points of the even members of the normal fatty acid salts or of the corresponding amides of *n*-octadecylamine against the number of carbon atoms in the acid radicals. The minimum occurs at six carbons in the case of the salts and at four in the case of the amides. An insufficient number of odd-chained members of these series was prepared to permit a similar comparison.

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[18] Hoyt, Doctoral Thesis No. 560, Iowa State College (1940), p. 78.

[19] Ref. 18, p. 83.

[20] Shinozaki and Kubo, *J. Agr. Chem. Soc. Japan.*, 13, 1 (1937); [*C. A.* 31, 3002 (1937)].

[21] French Patent 735,647 [*Chem. Zentr.*, 104, 1224 (1933)].

[22] Turpin, *Ber.*, 21, 2486 (1888).

[23] Jeffreys, *Am. Chem. J.*, 22, 22 (1899).

[24] Naegeli, Grüntuch-Jacobsen and Lendorff, *Helv. Chim. Acta*, 12, 227 (1929).

[25] de'Conno, *Gazz. chim. ital.*, 47, I, 93 (1917); [*C. A.*, 12, 1172 (1918)].

[26] Gilman and Ford, *Iowa State Coll. J. Sci.*, 13, 135 (1939).

[27] Robertson, *J. Chem. Soc.*, 115, 1210 (1919).

TABLE I

SALTS AND AMIDES OF *n*-OCTADECYLAMINE

| ACID | SALT | | | | | AMIDE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M. P. °C. | Mixed M. P. °C. | Pctg. Yield | Anal. Pctg. N | | M. P. °C. | Mixed M. P. °C. | Pctg. Yield | Anal. Pctg. N | |
| | | | | Calcd. | Found | | | | Calcd. | Found |
| Formic | 78.5–79.5 | | 87 | 4.44 | 4.44 | 68–68.5 | 67–76 | 95 | 4.70 | 4.50 |
| Acetic | 84.5–85 | | 87 | 4.27 | 4.28 | 78–78.5§ | 74–77 | 90 | 4.50 | 4.45 |
| Propionic | 78.5–79* | | 82 | 4.08 | 3.92 | 77–77.5* | 74–77 | 85 | 4.31 | 4.26 |
| *n*-Butyric | 71 –71.5 | | 81 | 3.98 | 3.79 | 76–76.5 | 75–76 | 90 | 4.14 | 4.12 |
| *n*-Valeric | 60 –61 | | 70 | 3.78 | 3.57 | 76–76.5 | 75–77 | 90 | 3.98 | 4.05 |
| Caproic | 55 –56 | 54–57 | 80 | 3.64 | 3.73 | 78–78.5 | 73–76 | 90 | 3.81 | 3.91 |
| Caprylic | 57.5–58 | | 94 | 3.38 | 3.23 | 79–79.5 | 77–80 | 85 | 3.65 | 3.38 |
| Capric | 62 –62.5 | | 84 | 3.17 | 3.28 | 83–83.5 | 81–84 | 91 | 3.30 | 3.06 |
| Lauric | 68 –69 | | 88 | 2.98 | 3.08 | 87.5–88‖ | 85–87 | 85 | 3.10 | 2.98 |
| Myristic | 78 –78.5 | | 91 | 2.82 | 3.06 | 89–89.5‖ | 88–90 | 85 | 2.95 | 3.10 |
| Palmitic | 85 –85.5 | 84–88 | 95 | 2.67 | 2.90 | 91.5–92‖ | 91–94 | 90 | 2.76 | 2.96 |
| Stearic | 89.5–90.5 | | 93 | 2.53 | 2.30 | 95.5–96‖ ¶ | | 92 | 2.62 | 2.72 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Benzoic ..... | 65 -66 | | 95 | 3.59 | 3.45 | 85.5-86‖**†† | | 95 | | |
| Anthranilic ..... | 92.5-93.5 | | 92 | 6.90 | 6.72 | ‡‡ | | | | |
| α-Furoic ..... | 91 -92 | | 95 | 3.68 | 3.56 | 79.5-80.5§§ | | 93 | 3.86 | 3.91 |
| Cinnamic ..... | 80.5-81.5 | | 93 | 3.36 | 3.27 | 90-91 | | 93 | 3.51 | 3.50 |
| Salicylic ..... | 73.5-74† | | 97 | 3.44 | 3.32 | 74.5-75.5† ‖ ‖ | | 25 | 3.61 | 3.82 |
| Phenylacetic ..... | 85 -85.5 | | 90 | 3.51 | 3.42 | 94.5-95 | | 75 | 3.62 | 3.60 |
| Oxalic ..... | 203 -205‡ | | 64 | 4.45 | 4.40 | 120-121 | | 61 | 4.73 | 4.64 |
| α-Naphthoic ..... | 109 -110 | | 92 | 3.17 | 3.14 | 89.5-90¶¶ | | 85 | 3.32 | 3.48 |
| 2-Dibenzofuran-carboxylic ..... | 88 -89‡ | | 71 | 2.91 | 3.04 | 118-118.5 | | 85 | 3.02 | 3.04 |

* A mixture of N-*n*-octadecylammonium propionate and N-*n*-octadecyl propionamide melted at 74-77°.
† A mixture of N-*n*-octadecylammonium salicylate and N-*n*-octadecyl salicylamide melted at 70-73°.
‡ Ethyl alcohol was the solvent used in preparing this salt.
§ Prepared previously (ref. 15).
‖ Also prepared directly from acid and amine without isolation of salt (ref. 13).
¶ Prepared previously from acid chloride, Hoyt gives m.p. of 96-97° (ref. 19).
** Prepared previously from acid chloride, Hoyt gives m.p. of 85-87° (ref. 18).
†† Also prepared from benzoyl chloride and amine; m.p. and mixed m.p., 85.5-86°.
‡‡ No pure product was obtained upon attempted dehydration of salt.
§§ Also prepared from furoyl chloride and amine; m.p. and mixed m.p., 79.5-80.5°.
‖ ‖ Heating of N-*n*-octadecylammonium salicylate effected partial decomposition and the odor of phenol was detected.
¶¶ Also prepared from α-naphthoyl chloride and amine; m.p. and mixed m.p. 89-90°.

## TABLE II

### Salts and Amides of *n*-Dodecylamine

| Acid | Salt | | | | | Amide | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M. P. °C. | Mixed M. P. °C. | Pctg. Yield | Anal. Pctg. N Calcd. | Found | M. P. °C. | Mixed M. P. °C. | Pctg. Yield | Anal. Pctg. N Calcd. | Found |
| Acetic | 67 -68 | | 90 | 5.71 | 5.54 | 53-54‡ | 50-53 | 90 | 6.15 | 5.85 |
| *n*-Propionic | 56 -57* | | 64 | 5.41 | 5.25 | 53-53.5* | | 77 | 5.80 | 5.60 |
| Lauric | 72 -73 | 67-68 | 91 | 3.63 | 3.59 | 77-77.5§ | 77-81 | 90 | | |
| Myristic | 72.5-73 | | 93 | 3.39 | 3.38 | 84-85§ | | 85 | | |
| Palmitic | 72 -73 | 68-70 | 93 | 3.17 | 2.99 | 82.5-83§ | 82-84 | 85 | | |
| Stearic | 69 -70† | 69-71 | 92 | 2.93 | 2.93 | 85-85.5§ | 82-84 | 85 | | |
| α-Furoic | 72.5-73 | | 91 | 4.73 | 4.48 | 57-58‖ | | 88 | 5.03 | 5.28 |
| Phenylacetic | 68.5-69.5 | | 65 | 4.36 | 4.06 | 79-79.5¶ | | 80 | 4.62 | 4.59 |
| α-Naphthoic | 114 -115 | | 73 | 3.97 | 3.73 | 71-72 | | 20 | 4.13 | 3.96 |
| Chloroacetic | 65 -66 | | 75 | 5.00 | 4.86 | (**) | | | | |
| 2-Dibenzofuran-carboxylic | 87.5-88.5 | | 75 | 3.52 | 3.40 | 112-113 | | 80 | 3.70 | 3.96 |
| Cinnamic | 53.5-55 | | 75 | 4.20 | 4.23 | 74-74.5 | | 80 | 4.45 | 4.78 |

*Mixed m.p. of N-*n*-dodecylammonium propionate and N-*n*-dodecyl propionamide was 48-54°.
† Mixed m.p. of N-*n*-dodecylammonium stearate and stearic acid was 59-67°.
‡ Prepared previously (ref. 16 and ref. 13, p. 100).
§ Also prepared directly from acid and amine without isolation of salt. The analyses are reported elsewhere (ref. 13).
‖ Also prepared from furoyl chloride and amine; m.p. and mixed m.p. 57-58°.
¶ Prepared previously (ref. 17).
** Preparation is in progress.

A long series of fatty acid diamides of ethylene diamine has been prepared by Tucker,[28] who heated the ethyl esters of the fatty acids (from $C_2$ to $C_{17}$, except $C_9$) with ethylene diamine. Details for the preparation of hexamethylene distearamide and hexamethylene dilauramide are given in a U. S. Patent.[29] Such compounds may be used as high-melting waxes, plasticizers, or as "slip-promoting agents" to prevent sheet materials from sticking together. We have prepared the salt of oxalic acid and octadecylamine and found that this substance is also very high melting (203-205°) and exhibits the "feel" of talc.

In the preparation of N-substituted amides of long-chained amines we have found that the direct formation from the acid and amine takes place very smoothly and in very good yields. The intermediate salts are usually readily isolated and generally lend themselves to ready purification from excess of either of the starting materials. Best results are obtained, however, if the acid and amine are mixed in exact molecular proportions. The isolation of the salt is not essential, and by careful measuring of molecular quantities of both acid and amine Harber[30] has shown that good yields of pure amides may be obtained by simply heating the reactants together in an atmosphere of nitrogen. The inert atmosphere is used to reduce darkening due to air oxidation at the temperature necessary for dehydration of the amine salt.

The long-chained amine salts were readily prepared by warming the amine with a carboxylic acid in a suitable solvent. Petroleum ether (b. p., 60-68°) was the solvent most generally used; however, ethyl alcohol served very well in the case of less soluble salts. No ester formation was observed. The amine salts are usually considerably less soluble in cold petroleum ether than the long-chained amines or fatty acids and thus are easily separated from these impurities. Certain carboxylic acids, however, are quite insoluble in petroleum ether and dissolve only upon addition of the amine to the petroleum ether mixture. The acid dissolves only to the extent to which it reacts to form the salt, and hence excess acid may be filtered from the hot solution. Upon cooling, the pure salt crystallizes from the filtrate.

## EXPERIMENTAL PART

### PREPARATION OF AMINE SALTS

The following procedure illustrates the method that has been found satisfactory for a large number of amine salts:

To a solution of 2.69 g. (0.01 mole) of *n*-octadecylamine in 100 cc. of petroleum ether (b. p., 60-68°) was added 2.84 g. (0.01 mole) of stearic acid. The mixture was warmed until all had passed into solution. The warm mixture was filtered and the filtrate allowed to cool slowly until the salt crystallized out. The product was filtered and dried on a Büchner fun-

[28] Tucker, *J. Am. Chem. Soc.*, 57, 1989 (1935).
[29] U. S. Patent 2,132,388 [*C. A.*, 33, 174 (1939)].
[30] Harber, Doctoral Thesis No. 590, Iowa State College (1940).

nel. The yield of N-*n*-octadecylammonium stearate was 5.15 g. (93 per cent). Successive recrystallizations from petroleum ether and alcohol failed to raise the melting point (89.5-90.5°). The salt was analyzed by the micro Kjeldahl method.

### PYROLYSIS OF AMINE SALTS TO AMIDES

Conversion of the amine salts to amides could usually be effected by simply heating the salt in a nitrogen atmosphere between 225-250° for from fifteen to thirty minutes. Water was smoothly evolved under these conditions and very little discoloration of the product was observed. The amide was purified by recrystallization from petroleum ether or alcohol and in most cases was obtained in practically theoretical yield. A typical procedure is illustrated in the preparation of N-*n*-octadecyl stearamide:

N-*n*-Octadecylammonium stearate (2 g.) was heated in a nitrogen atmosphere on a Wood's metal bath between 225-250° for fifteen minutes. The cooled product weighed 1.95 g. (98 per cent) and melted constantly at 94.5-95° after two recrystallizations from petroleum ether.

The melting points, yields, and analyses of the salts and amides of *n*-octadecylamine and *n*-dodecylamine are given in Tables I and II, respectively. Mixed melting points of contiguous members whose melting points were within 5° are also given. As the depressions are generally slight, this method is of questionable value in differentiating these substances. The limitations of mixed melting point determinations in differentiating successive members in a series of long-chained derivatives has been noted previously by Gilman and Ford,[31] by McCorkle,[32] and others.

ACKNOWLEDGEMENTS: The author is grateful to Professor Henry Gilman for guidance and to Dr. A. W. Ralston for helpful suggestions as well as for liberal supplies of some of the initial materials.

## SUMMARY

The preparation of a number of aliphatic and aromatic carboxylic acid salts and amides of *n*-dodecylamine and *n*-octadecylamine has been described. The method of amide formation is more direct than those generally used inasmuch as the carboxylic acid itself rather than a derivative of the acid reacts directly with the amine.

The melting point curves for a series of salts and a series of amides of *n*-octadecylamine and normal fatty acids show a minimum point at $C_6$ in the case of the salts and at $C_4$ in the case of the amides.

[31] Gilman and Ford, *Iowa State Coll. J. Sci.*, **13**, 135 (1939).
[32] McCorkle, Doctoral Thesis No. 481, Iowa State College (1938).

# LASIUS (ACANTHOMYOPS) PLUMOPILOSUS, A NEW ANT WITH PLUMOSE HAIRS FROM IOWA

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**Lasius (Acanthomyops) plumopilosus** sp. nov.

FEMALE

Length, 4.6 mm.

Head excluding the mandibles very nearly as broad (.97 mm.) as long (1.00 mm.), with very feebly excised posterior border and slightly convex sides. Eyes convex, hairy. Mandibles 6-dentate. Head without a frontal furrow. Scapes nearly reaching the posterior corners of the head, gradually thickened distally. Funiculi less incrassated than in *claviger*, joints 2-10 broader than long, the penultimate joints very little broader than long. Thorax 1.70 mm. long, .87 mm. wide (across the wing insertions); mesonotum and scutellum forming a nearly flat surface above. Epinotum with a very short base and steep declivity. Petiole small, its superior border faintly notched and blunter than in *L. (A.) claviger* and *interjectus*, the scale a little less than two-thirds as high as the epinotum. Gaster long, the sides subparallel. Head and body in profile formed about the same as in *claviger*. Tibiae and femora flattened, fore femora about 3 times, middle and hind femora about 4 times, as long as broad. Wings 5.4 mm. long.

Whole body strongly shining with pilosity and pubescence arising from punctures. Mandibles finely striate.

Pilosity extraordinary, nearly all of the erect hairs plumose or at least branching at the tip. Plumose hairs best developed on the head, thorax, petiole, and mid-dorsal region of the first gastric segment. Pinnules of the hairs in no case covering more than the distal half of a hair, in many cases not covering more than the distal one-fourth or one-sixth, and in many cases on the gaster merely 3, 4, or 5-branched at the tip. Number of pinnules on well-developed plumose hairs about 8-15. Hairs present on the clypeus, on the front in a fringe pointing inwardly, on the vertex, occiput, gula, pronotum, prosternum, on the mesonotum in a fringe pointing inwardly, on the scutellum, mesepisternum, mesosternum, metanotum, epinotum, and petiole; very numerous on the gaster including the ventral side of the first segment (bases of contiguous hairs on the gaster about .04-.10 mm. apart). Succeeding gastric segments on the ventral side with a row or two of hairs along their posterior margins. Also

[1] The writer desires to express his appreciation to Dr. H. H. Knight, Professor of Entomology, Iowa State College, for much assistance and encouragement; to Dr. M. R. Smith, Associate Entomologist, Bureau of Entomology and Plant Quarantine, Washington, D. C., for kindly checking the writer's determination of this species as new; and to Judson U. McGuire, Jr., for drawing the figures.

present although much smaller and fewer on the coxae, trochanters, and fore femora. Length of hairs about .16-.20 mm.; of well-developed pinnules, about .03-.04 mm. Pubescence rather sparse on most parts of the body, denser on the legs, antennae, ventral surface of the pedicel, and base of gaster. Second and succeeding gastric segments with bands of very fine, short pubescence on the anterior margins, changing posteriorly into sparser, longer pubescence. Pubescence on the genae and gula very long, strong, sparsely set, and subappressed.

Color much darker brown than in the females of *interjectus* and *latipes,* the head and thorax appearing brownish black to the naked eye; not as dark, however, as in some specimens of *claviger.*

WORKER

Length, 3.2 mm.

Head, excluding the mandibles very nearly as broad (.93 mm.) as long (.94 mm.), with a very slightly excised posterior border and convex sides; widest across the middle of the head. Mandibles 6-dentate. Eyes hairy, slightly convex, with about 45 ommatidia. Scapes not quite reaching the posterior corners of the head. Funiculi incrassated, joints 2-9 at least slightly broader than long, joint 10 about as broad as long. Thorax with the shape of *claviger;* 1.16 mm. long and .58 mm. wide (across the pronotum); meso-epinotal suture relatively shallow. Petiole not quite as high as the epinotum, the superior border notched and about as blunt as in *claviger;* having about the same shape as in *claviger* when seen from behind; anterior surface convex, posterior flat. Femora and tibiae flattened, fore femora about 3 times, middle and hind femora about 4 times, as long as broad.

Whole body very shining. Mandibles finely striate.

Many of the erect hairs plumose or branching at the tip, although not as much as on the female. Plumose hairs best developed in a row on the occiput, on the pronotum, mesonotum, epinotum, and mid-dorsal region of the first gastric segment. Hairs present whether plumose or not on the clypeus, front, occiput, gula, pronotum, mesonotum, epinotum, petiole, coxae, and fore femora; numerous on the gaster including the ventral side of the first segment. Succeeding segments on the ventral side with a row or two of hairs along their posterior margins. Length of hairs about .10-.15 mm. Pubescence sparse, denser on the legs, coxae, and antennae. Second and succeeding gastric segments with bands along the anterior margins of very fine, short pubescence. Pubescence on the genae and gula very long and strong.

Color sordid yellow, mandibles and funiculi more brownish.

MALE

Length, 3.2 mm.

Head excluding the mandibles little broader (.80 mm.) than long (.75 mm.), broader behind than in front, with rather convex posterior border and very slightly convex sides. Eyes hairy, very convex, situated

on the sides halfway between the corners of the head. Mandibles with 6 teeth, but counting from the basal border, only numbers 4, 5, and 6 on the right mandible and numbers 3, 5, and 6 on the left mandible very distinct. Frontal furrow distinct. Antennae long and slender, the scapes surpassing the posterior corners of the head. Funiculi with first joints swollen, other joints cylindrical, joints 2-11 very gradually increasing in length. Thorax 1.42 mm. long, through the wing insertions less broad (.74 mm.) than the head. Declivity of the epinotum more sloping than in the female. Petiole with a very sharp border, its anterior surface slightly concave and its posterior surface slightly convex. Wings 4.2 mm. long, discal cell absent.

Less shining than the worker and female; the head and thorax finely punctate and subopaque.

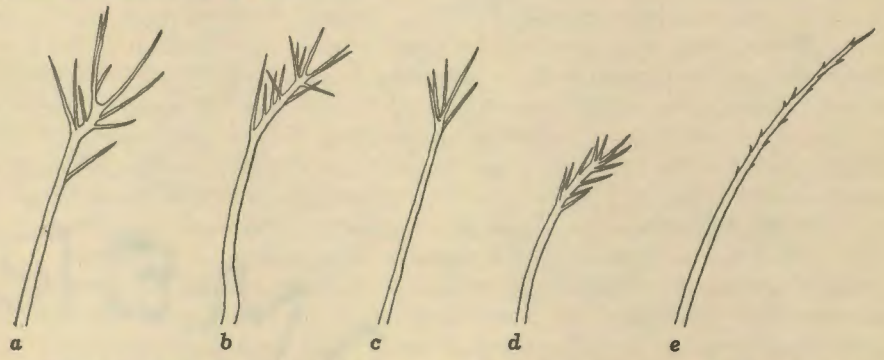


FIG. 1. *L. (A.) plumopilosus* hairs: *a* and *b*, from mesonotum of female; *c*, from abdomen of female; *d*, from pronotum of worker. *L.(A.) claviger* hair: *e*, from thorax of female.

Hairs sparser, and more slender than in the worker and female, a little longer (about .11-.16 mm.) than in the worker; not plumose generally, although several hairs on the clypeus, vertex, occiput, mesonotum, and scutellum branching at the tip. Pubescence more sparse than in the female and not as varied in size.

Color very dark brown appearing black to the naked eye. Head and mesonotum very nearly black. Funiculi and legs infuscated yellow.

### VARIATION IN TYPE MATERIAL

Both females and workers vary somewhat in the plumosity of the hairs, in the arrangement of the hairs, and in the location of the most plumose ones. In general, however, they are as described. There is little difference in size in either females or workers. Several of the paratype females, however, have the gaster wider and larger than in the holotype. The discal cell of the fore wing is very variable, being either present, incomplete, or absent. The holotype has the discal cell present on the left wing, absent on the right. The shape of the petiole varies also; one female seems to be without even a slight notch on the superior border, which is evenly rounded above.

Described from a series of 8 alate females, 6 workers, and 1 male taken from a colony nesting under a hillside rock at Backbone State Park, Iowa, September 13, 1940.

Holotype female, 1 paratype female, 2 paratype workers, and the paratype male are to be deposited in the National Museum, Washington, D. C.; 3 paratype females and 2 paratype workers are to be deposited in the Iowa State College collection; and 3 paratype females and 2 paratype workers are to remain in the author's collection.

This species appears to be closely related to *L. (A.) claviger* from which it can be separated very definitely by the following points: (1) the highly specialized plumose hairs of the female and worker, (2) the smaller size of the worker and the decidedly smaller size of the female and male, (3) the 6-dentate mandibles of the male, and (4) the much less incrassated funiculi of the female, the penultimate joints being only about 1.1 times as broad as long, whereas in the females of *claviger* that the writer has measured the penultimate joints are 1.3 to 1.4 times as broad as long. Other differences include: (1) the very abundant hairs on the gaster of the female, (2) the proportionately shorter hairs on the female and worker, (3) the more highly specialized pubescence on the female and worker, (4) the less flattened femora and tibiae of the female, and (5) the proportionately more slender body of the female and male.

Two other species of the subgenus *Acanthomyops, L. (A.) interjectus coloradensis* Wheeler and *L. (A.) interjectus mexicanus* Wheeler, also have very small females 5 mm. long or less, very small workers about 3 mm. long, relatively short antennae, and males with dentate mandibles. From Wheeler's descriptions of *coloradensis* and *mexicanus, plumopilosus* can probably be distinguished by at least the following points: (1) the highly specialized plumose hairs on the female and worker, (2) the edentate basal border of the mandibles in the female and worker, (3) the shorter antennae, the scapes not reaching the hind corners of the head, (4) the much more numerous hairs, especially on the gaster of the female, than on *mexicanus,* at least, (5) the greatly varied pubescence which is also decidedly less profuse than on *mexicanus,* and (6) the more flattened legs of the female and worker. Future comparisons of these two forms and especially of *coloradensis* with *plumopilosus* will perhaps throw more light on the latter's affinities.

As far as the writer has been able to ascertain, this is the first recorded instance of plumose hairs in the Formicidae. At least this type of pilosity is very rare among ants. However, examination under the highest power of the binoculars shows some females of *L. (A.) interjectus, claviger,* and *latipes* to have hairs with a very poorly developed type of plumosity consisting of short, very tiny, rather suberect pinnules which tend to be scattered along the length of the hair rather than collected distally. Short, very tiny pinnules can sometimes be seen on the hairs of the workers of *interjectus, claviger,* and *latipes,* also. The author has not had the opportunity of examining specimens of *claviger* subsp. *subglaber, occidentalis,*

*murphyi*, or any of the subspecies of *interjectus* and so cannot make any statements regarding them.

Many of the North American *Lasii* of the subgenera *Acanthomyops* and *Chthonolasius* are considered to be temporary social parasites of the varieties of *L. niger*. The very small size and extraordinary pilosity of the females of *plumopilosus* seem to suggest, however, that this species may be a more highly specialized parasite than other species of this genus.

# CONCERNING SOME HALOBATINIDS FROM THE WESTERN HEMISPHERE (HEMIP. GERRIDAE)

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Received February 1, 1941

The present paper contains the descriptions of two new Halobatinae from Panama and Brazil and notes on some other gerrids from the Western Hemisphere. The types are in the collection of the authors.

### Halobatopsis platensis (Berg)

*Halobates platensis* Berg, Anales Soc. Cient. Arg., 8, 1879, p. 24 (Hemip. Arg., p. 183).
*Halobatopsis platensis* Drake and Harris, Notas Museo La Plata, III, 1938, p. 20, fig. 1.

This species shows considerable variation in color. It is known from Argentina and Brazil. Heretofore, only the apterous form was known.

*Winged form:* Markings on head same as in apterous. Pronotum large, longer than wide, subtriangularly produced behind, convex above, the apex broadly rounded; largely black, with an irregular discal spot on anterior lobe and two large patches on hind lobe testaceous to brownish testaceous. The somewhat rectangular spot on the front of the pronotum connected at its outer hind angles to the lateral pale stripe by a fine, sometimes incomplete, curved pale line. Hemelytra black, opaque, the veins darker and hairy. In four of the specimens the wings are broken off at the transverse suture.

*Morphotype,* Belo Horizonte, Minas Geraes, Brazil, Oscar Monte, in authors' collection.

### Halobatopsis delectus, sp. nov.

*Apterous form:* Similar to *H. spiniventris* Drake and Harris, but readily distinguishable by the raised knob within near the basal fourth of the fore femora, the sinuate and moderately swollen fore tibiae near the middle, and the distinctly sharper, narrower and more deeply excavated notch on the hind margin of the last venter of the male. The posteriorly projecting spine in the notch of the last venter of male testaceous, moderately prominent, projecting a little beyond hind margin of venter; first genital segment testaceous, longitudinally ridged along median line; the last segment embrowned distally, there clothed with brown hairs. Last segment of venter long, a little longer than two preceding segments, very broadly, roundly excavated behind. Fore legs brownish black, the femora curved, with a broad, testaceous stripe along the greater portion of outer surface. Middle and hind legs brownish black. First genital above notched behind, dark brown distally.

In both sexes the second antennal segment a little longer than in *spiniventris,* connexiva of female with tufts of dark hairs as in *spiniventris.* Last venter in female as long as three preceding. The fore legs not modified as in male. Color and markings very similar to *spiniventris* (Drake and Harris, Rev. Ent., VII, 4, 1934, p. 358, fig. 2c). Fore legs of female not modified, the hind femora with hairs within as in *spiniventris.*

Length, 4.00-4.60 mm.; width, 1.75-2.00 mm.

*Holotype* (male), *allotype* (female), and 3 *paratypes,* Belo Horizonte, Minas Geraes, Brazil, collected by Oscar Monte.

This is the fourth member of the genus and the only one having modified front femora and tibiae in the male. The peculiar spine of the last venter of male is dark brown in *spiniventris* and testaceous in *delectus.*

### Telmatometra whitei Bergroth

*Telmatometra whitei* Bergroth, Ohio Nat., VIII, 1908, p. 374; Drake and Harris, Rev. Ent., VII, 1937, p. 360, fig. 2a.

Originally described from 5 winged males and females from Escuintla, Guatemala. Many apterous specimens and 2 winged examples are at hand from Barro Colorado Island, Canal Zone, Gatum and Panama City, Panama, collected by C. J. Drake; Mayaguez, Puerto Rico, H. D. Tate; Moralies, Guatemala, J. J. White; and Punta Gorda, British Honduras, J. J. White. The color markings exhibit considerable variation in a series of specimens taken at one station in the wide reaches of a stream near old Panama City. The black lateral stripes of the pronotum vary much in width. The median stripe may be entirely wanting or may vary from a short basal stripe to a complete longitudinal, median stripe, which also varies considerably in width. The forms with a total median line agree perfectly with Esaki's *T. ujhelyii.* Specimens of typical *whitei* and *ujhelyii* were taken by C. J. Drake in the same school in wide reaches of a stream at old Panama City and in a quiet pool of a small stream at Barro Colorado Island, Canal Zone, Panama.

### Telmatometra whitei ujhelyii Esaki

*Telmatometra ujhelyii* Esaki, Ann. Mus. Nat. Hung., XXIII, 1926, p. 133, fig. 4.

This form is treated here as a color variety of *whitei* Berg. There are no antennal, genital or other structural differences which will distinguish the two forms. Winged specimens of *whitei* agree perfectly with Esaki's description and figure. The extreme color differences of apterous forms seem to warrant the retaining of *ujhelyii* as a color variety.

### Telmatometra rozeboomi Drake and Harris

*Telmatometra rozeboomi* Drake and Harris, Rev. de Entomologia, VII, 1937, p. 358, fig. 2b.

One male, taken in the wide reaches of stream near old Panama City,

Pan., Feb. 10, 1939, C. J. Drake. This species is considerably smaller, very differently colored and has also a great difference in genital character from *whitei*. It is smaller and differs markedly in external genital structure from *panamensis*, n. sp.

**Telmatometra panamensis**, sp. nov.

*Apterous form:* Moderately elongate, yellowish brown, with prominent black-fuscous markings. Head with sides and lateral margins above (widening posteriorly) fuscous-black, the broad central portion (narrowing posteriorly) and a very narrow margin behind testaceous. Eyes blackish. Rostrum long, extending about one-third of its length beyond mesosternum, testaceous, the distal third blackish. Antennae long, slender, brownish black; antennal proportions—I, 16; II, 9; III, 22; IV, 20. Pronotum nearly twice as long as broad, broadly impressed on the disc, testaceous above, the sides and a very narrow border in front and behind black-fuscous; mesonotum long, dorsal surface and a wide enclosed strip on each side testaceous, the rest, including a narrow front margin and a short, median, basal streak black-fuscous; mesonotum with a large spot on each side testaceous, the median black stripe becoming wider posteriorly. Abdomen above testaceous, the first two segments, sutures between segments, and narrow outer margins of connexiva black-fuscous. Body beneath testaceous, anterior legs mostly dark brown, the femora above with a broad, longitudinal, testaceous stripe; tibiae also with a pale stripe along dorsal side. Middle and hind legs dark brown, the femora beneath testaceous.

*Winged form:* Pronotum very large, long, convex above, nearly triangular behind, rounded at apex, testaceous, the sides very broadly margined with blackish fuscous, narrow in front, a very large broad, triangular spot on the disc extending to the hind margin as a median line dark fuscous. Hemelytra black-fuscous, the veins hairy, darker and prominent, broken off on the five examples at the suture.

*Male:* Last venter long, as long as three preceding, the hind margin broadly concavely rounded; first genital segment beneath long, flat, hairy, sharply triangularly notched behind; the second segment short, rounded behind.

*Female:* Broader than male. Last venter very long, longer than three preceding segments, very broadly and deeply excavated behind; first genital segment long, broad, flattened beneath, the terminal segment very short.

Length, 3.95 mm.; width, 1.25-1.55 mm.

*Holotype* (male), *allotype* (female), and winged *paratypes*, Canal Zone, Panama, Feb. 10, 1939, C. J. Drake.

This species may be easily distinguished from its congeners by the color markings, the last venter, and male genital segments.

CHARMATOMETRA BAKERI (Kirkaldy)

*Brachymetra bakeri* Kirkaldy, Entom. XXXI, 1898, p. 101.
*Charmatometra bakeri* Kirkaldy, Ann. Ent. Soc. Belg., XLIII, 1899, p. 509.

Recorded in the literature from Ecuador and Colombia. The writers also have several specimens from Venezuela. It is the largest halobatid recorded from the Western Hemisphere. The macropterous form is unknown.

CYLINDROSTHETHUS PALMARIS Drake and Harris

*Cylindrosthethus palmaris* Drake and Harris, Ann. Carn. Mus., XXIII, 1934, p. 222.

Many apterous specimens, Trinidad, B. W. I., Oct. 27, 1938, C. J. Drake. This species was found in considerable numbers in the wide reaches of small streams at many different points in the island. It is a very active species and moves rapidly over the surface of the water when disturbed. This species and the following belong to the subfamily Gerrinae.

CYLINDROSTHETHUS LINEARIS Erichson

*Cylindrosthethus linearis* Erichson, in Schmburgh's Fauna Brit. Guiana, III, 1848, 18, p. 614; Drake and Harris, Ann. Carnegie Mus., XXIII, 1934, p. 220.

One male, Trinidad, Bolivia, Collection of M. S. Pennington. Known heretofore only from British Guiana and Rio de Janeiro, Brazil.

# SOME NEW SPECIES OF MIRIDAE (HEMIPTERA) FROM CHINA

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The following descriptions of eleven new species and one new variety are based on a small collection of Chinese mirids in the possession of the United States National Museum and some specimens kindly sent the writer by Dr. Gaines Liu, the Tea Experiment Station, Kweichow, China. The types are deposited as indicated under each species.

**Bryocoris convexicollis sp. nov. (Fig. 1)**

FEMALE. Body elongate, length 4 mm., width 1.5 mm., clothed with pale fine simple pubescence, color principally black, shining.

*Head:* vertical, small, width across eyes 0.6 mm., length 0.6 mm.; vertex convex, with 0.35 mm., very tenuously marginate, lateral margins near eyes pale, longitudinal median sulcus shallow and broad; frons convex, depressed immediately above base of clypeus; clypeus prominent; juga flat, slightly paler; genae medium; gula very short. Eyes small, almost contiguous with apex of pronotum. Rostrum flavous, short, not reaching apex of mesosternum.

*Antennae:* inserted at middle of interior margin of eyes, linear, fuscous; segment I, length 0.34 mm., slightly paler; II, length 1.12 mm., slightly thickened at apex, apex nearly as thick as I; III, length 0.5 mm.; IV, length 0.43 mm., the last two segments more slender than II.

Pronotum, length 0.74 mm., width at base 1.25 mm., at apex 0.45 mm., impunctate, strongly convex posteriorly; collar, base before scutellum and posterior angles flavous; posterior and lateral margins straight, collar as thick as antennal segment I, calli hardly distinguished, slightly impressed posteriorly. Mesoscutum covered. Scutellum, length 0.43 mm., width at base 0.6 mm., moderately tumid, lateral margins widely pale. Xyphus very concave, lateral margins elevated. Mesosternum somewhat convex, with middle part depressed; ostiolar peritreme creamy pale, anterior margin strongly elevated. Venter ferruginous-fuscous.

*Hemelytra:* pale, semitransparent; embolar margin narrowly, apex of corium broadly, and apical half of cubital vein, base of clavus broadly, interior margin and commissure narrowly, apex of cuneus, membrane with apical margin very narrowly and veins, fuscous. Corium, length 1.51 mm., lateral margin slightly curvate. Cuneus, length 0.95 mm., width at base 0.6 mm.

*Legs:* slender, moderately long, flavous, apical third of femora, tibia, apex of tarsi and claws lightly ferruginous.

[1] Grateful acknowledgement is made to Dr. H. H. Knight for valuable suggestions and assistance.

Allied to *B. pteridis* Fall. but differs in the larger size, more convex pronotum, and the coloration of antennae, clavus, scutellum and hind femora.

*Holotype:* ♀ ,Omei San, Szechuan, July, 1932, collected by G. Liu, in the author's collection.

**Deraeocoris nigropectus** sp. nov.

FEMALE. Body oblong, length 4.16 mm., width 2.04 mm.; above glabrous, ochraceous with dark markings, coarsely nigropunctate except on head and scutellum; beneath black, clothed with fine golden pubescence.

*Head:* slightly inclining, smooth, shining, lightly fulvous, length 0.65 mm., width 0.87 mm.; vertex, width 0.39 mm., very tenuously marginate, margin narrowly black; clypeus prominent, confluent with frons, fuscous at apex, Rostrum, length 1.52 mm., reaching apex of intermediate coxae, segment I attaining anterior margin of xyphus, segment IV fuscous.

*Antennae:* black, sparingly clothed with fine silvery hairs; segment I, length 0.43 mm., width 0.17 mm.; II, length 1.06 mm., slightly incrassate at apex, somewhat paler at basal half; segment III and IV missing.

Pronotum, length 1.10 mm., width at base 1.70 mm., at apex 0.68 mm., coarsely punctate; a large black spot on each side of disk confluent with each other anteriorly, thus forming an incomplete longitudinal median line; lateral margins straight, ecarinate, posterior margin somewhat rounded; collar, thickness 0.13 mm., shining. Scutellum, width at base 0.76 mm., length 0.49 mm., impunctate, black, lateral margins widely flavous. Xyphus with disk tending to dark, planate, sides elevated. Sternum dark, ostiolar peritreme pale. Venter uniformly black, clothed with fine golden hairs.

*Hemelytra:* width 1.04 mm., length 3.16 mm., coarsely black punctate; embolar margins moderately curvate, length 2.04 mm.; cuneus declivent, length 0.65 mm., width at base 0.74 mm., apex fuscous and smooth.

*Legs:* somewhat cylindrical; femora with two subapical fuscous rings; tibia with base, apex, and a wide ring at middle fuscous, weakly spinose; tarsi fuscous at apex, claws dark.

Allied to *D. orientalis* (Dist.) and *D. signatus* (Dist.) but distinguished from the former by the somewhat unicolorous hemelytra and the uniformly black antennal segment I, and from the latter by the shorter antennal segment I and more coarsely punctate hemelytra.

*Holotype:* ♀ , Pingloo, Kwangsi, May, 1933; 1 ♀ Paratype, Taipingshien, Anhwei, Oct., 1932; both were collected by G. Liu, in the author's collection.

**Deraeocoris anhwenicus** sp. nov.

FEMALE. Body oblong, length 4.75 mm., width 1.95 mm., above glabrous, fulvous, coarsely fuscous punctate (except head and scutellum), beneath ferruginous.

*Head:* slightly inclining, length 0.61 mm., width 0.82 mm.; vertex, width 0.35 mm., immarginate; clypeus moderately prominent, confluent

at base. Eyes reddish. Rostrum, length 1.54 mm., surpassing middle of intermediate coxae, fuscous at apex, segment I barely surpassing base of head.

*Antennae:* concolorous with body; segment I, length 0.42 mm., thickness 0.07 mm.; II, length 1 mm., clothed sparingly with rather long silvery hairs, slightly incrassate and ferruginous at apex; III, a little more slender than II; IV missing.

Pronotum, length 1.04 mm., width at base 1.65 mm., at apex 0.61 mm., coarsely punctate, posterior margin slightly rounded, lateral margins straight, ecarinate, disk posteriorly moderately convex; calli smooth, lightly fuscous, more or less confluent; collar, thickness 0.07 mm., concolorous, shining. Scutellum, length 0.52 mm., width at base 0.78 mm., dark brown, lateral margins paler. Xyphus with sides elevated. Ostiolar peritreme flavous. Venter clothed with simple flavous hairs.

*Hemelytra:* fuscous-punctate, longly surpassing apex of abdomen; embolar margins moderately curvate, length 1.08 mm.; cuneus, length 0.65 mm., width at base 0.74 mm., apex smooth; membrane and veins concolorous.

*Legs:* testaceous, two subapical rings on femora, apex, sub-base and middle of tibia, apex of tarsi and claws, fuscous to blackish; tibia with very few testaceous spinules.

Allied to *D. nigropectus*, n. sp., but distinguished readily by the concolorous pectus, more or less unicolorous pronotum, immarginate vertex and sparingly spinulose tibiae.

*Holotype:* ♀, Taipingshien, Anhwei, Oct., 1932, collected by G. Liu, in the author's collection.

**Deraeocoris alticallus sp. nov**

FEMALE. Body oblong, length 5.15 mm., width 2.25 mm., tawny brown, glabrous, coarsely punctate except on head and scutellum.

*Head:* slightly inclining, length 0.71 mm., width 0.91 mm.; vertex, width 0.42 mm., very tenuously marginate; clypeus moderately prominent concolorous, confluent with frons at base. Eyes not contiguous with pronotum, fuscous, tinged with reddish. Rostrum, length 2.15 mm., barely reaching middle of hind coxae; segment I surpassing base of head, IV fuscous at apex.

*Antennae:* linear, flavous, clothed with pubescent hairs; segment I, length 0.65 mm., thickness 0.09 mm., cylindrical, tinged with reddish; II, linear or very slightly thickened at apex, length 1.91 mm., distinctly more slender than I, fuscous on apical fourth; III, length 0.82 mm., fuscous at extreme apex; IV, length 0.74 mm., slightly fuscous on apical third; the last two segments as thick as base of II.

Pronotum, length 1.17 mm., width at base 1.91 mm., at apex 0.78 mm., coarsely and rather closely punctate, lateral margins straight, posterior margin somewhat rounded, slightly marginate; calli strongly elevated, confluent with each other at middle, smooth; collar, thickness 0.06 mm., shining. Mesoscutum nearly covered. Scutellum, width at base 0.87 mm.,

length 0.74 mm., smooth, strongly convex. Xyphus with lateral margins elevated. Mesosternum concolorous, with a very tenuous longitudinal median line reddish. Venter slightly reddish, clothed with simple golden pubescence.

*Hemelytra:* longly surpassing apex of abdomen, coarsely punctate; embolar margins moderately curved, length 2.38 mm.; cuneus, length 0.74 mm., width at base 0.74 mm., declivent, smooth except on interior angle; membrane and veins fuscous, finely but distinctly rugulose.

*Legs:* cylindrical, ferruginous, base of femora flavous, spinules on tibia concolorous, tarsi flavous, claws fuscous.

Distinguished from *D. martini* (Put.) by the color of head, pronotum, and legs; from *D. anhwenicus,* n. sp. by the longer antennae, elevated calli, raised scutellum and very tenuously marginate vertex; and from *D. flavidus* Popp. by the larger size and wider vertex.

*Holotype:* ♀, Chungking, Szechuan, July, 1932, collected by G. Liu, in the author's collection.

**Deraeocoris montanus** sp. nov.

FEMALE. Body oblong, length 6.06 mm., width 2.42 mm., reddish to dark reddish brown, clothed both above and below with simple long flavous hairs; pronotum and hemelytra coarsely punctate.

*Head:* slightly inclining, length 0.56 mm., width 0.91 mm., concolorous with body, sometimes brownish, with clypeus and disk of frons tending to fuscous; vertex, width 0.43 mm., tenuously marginate; base of clypeus slightly discrete with frons. Rostrum, length 1.86 mm., reaching middle of intermediate coxae, darkened at apex.

*Antennae:* segment I relatively long, length 0.82 mm., thickness 0.09 mm., cylindrical, reddish to fuscous; II, length 2.08 mm., thickness 0.07 mm., linear, flavous, fuscous at apex; III, length 0.82 mm., thickness 0.05 mm., flavous, apex lightly darkened; IV, length 0.65 mm., fuscous apically.

Pronotum, length 1.08 mm., width at base 1.91 mm., at apex 0.78 mm., coarsely punctate, lateral margins straight, ecarinate, posterior margin slightly rounded; calli conspicuous, smooth, confluent; collar, thickness 0.06 mm., shining. Scutellum, width 0.95 mm., length 0.65 mm., impunctate, moderately convex, lateral margins widely pale. Xyphus with sides elevated, disk concave. Mesosternum flavous to piceous, ostiolar peritreme paler. Venter reddish to piceous.

*Hemelytra:* coarsely punctate, longly surpassing apex of abdomen; embolar margins moderately curvate, length 2.47 mm.; cuneus reddish to dark red, length 0.91 mm., width at base 0.65 mm., punctures obsolete; membrane fumose, finely rugulose, veins reddish to ferruginous.

*Legs:* flavous, apical half of femora, tibia (sometimes only at base), apex of tarsi and claws reddish to fuscous, tibiae without spines.

Allied to *D. pilipes* (Reut.) but distinguished by the slender and shining collar; the body is unicolorously reddish brown to fuscous and clothed all over with simple hairs.

*Holotype:* ♀, Mt. Omei, Szechuan, Sept. 21, 1938, collected by C. S. Tsi, in the author's collection. *Allotype:* ♂, Taichow, July 4, 1935, collector unknown, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* ♂, Taichow, July 8, 1935, collector unknown.

**Aretas chinensis sp. nov. (Fig. 2 a, b)**

MALE. Body oblong, length 3.03 mm., width 1.4 mm., yellow with red and black markings, clothed with fine concolorous simple hairs.

*Head:* vertical, width 0.68 mm., length 0.52 mm.; vertex, width at front margins of eyes 0.22 mm., obsoletely marginate; frons fuscous anteriorly, strongly convex; clypeus moderately prominent, fuscous at base, discrete with frons; juga and lora fuscous. Eyes large, reddish, coarsely granulate. Rostrum, length 0.87 mm., slightly surpassing hind margin of mesosternum.

*Antennae:* inserted at the middle of anterior margins of eyes; segment I, length 0.22 mm., thickness 0.11 mm., black; II, length 1.3 mm., cylindrical, as thick as I, black at extreme base; III, length 0.43 mm.; IV, length 0.4 mm.; both III and IV much more slender than II and fuscous at extreme base.

Pronotum, length 0.37 mm., width at base 1.05 mm., at apex 0.43 mm., basal margin slightly sinuate, lateral margins straight; a very fine transverse impressed line at anterior fifth forming a rather broad and indistinct collar, and another broader one at posterior two-fifths; a broad longitudinal band on each side reddish and tinged fuscous anteriorly. Mesoscutum broadly exposed, sides reddish. Scutellum, length 0.38 mm., width at base 0.46 mm., a longitudinal median line reddish. Ventral side unicolorous, genital segments fuscous. Male claspers as shown in the figure.

*Hemelytra:* embolar margins nearly straight; corium length 1.62 mm., base and apex red; clavus with basal half and a subapical spot red; cuneus, length 0.51 mm., width at base 0.33 mm., tinged reddish; membrane semitransparent, finely rugulose, veins reddish.

*Legs:* concolorous with body, posterior femora thickened, tibia sparsely but longly spinulose, spinules concolorous.

In the key by Dr. Knight (1937) this species runs down to *A. bakeri* Knight, but differs in color markings, size of body, and structure of the male genital claspers.

*Holotype:* ♀, Shin Kai Si, Mt. Omei, Szechuan, alt. 4,000 ft., date unknown, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C.

**Lygus minutus sp. nov.**

MALE. Body oval, length 3 mm., width 1.6 mm., yellowish (probably greenish when alive), subtranslucent, clothed with concolorous simple hairs.

*Head:* subvertical, length 0.70 mm., width 0.74 mm., apex below apical margin of eyes black, somewhat produced; vertex, width 0.32 mm., margination obsolete; clypeus not prominent, confluent with frons; lorum prominent; gula short; eyes large, hind margins forming straight line with base of vertex. Rostrum, length 1.51 mm., distinctly surpassing apex of hind-coxae, apex fuscous.

*Antennae:* inserted beyond the middle of interior margin of eyes; segment I, length 0.43 mm.; II, length 1.25 mm., black slightly incrassate at apex; segment III and IV missing.

Pronotum, length 0.74 mm., width at base 1.26 mm., at apex behind collar 0.64 mm., obsoletely punctate, basal margin moderately rounded, sides straight, calli small and smooth, collar as thick as base of second antennal segment. Mesoscutum covered. Scutellum, length 0.43 mm., width at base 0.56 mm., uniformly black. Venter ferrugino-fuscous.

*Hemelytra:* embolar margin slightly curvate, black; corium, length 1.51 mm., subapically fuscous; claval suture depressed, interior margin tinged fuscous; cuneus, length 0.43 mm., width at base 0.55 mm., basal half fuscous; membrane fumate, veins fuscous.

*Legs:* cylindrical, concolorous with body and devoid of fuscous markings, spinules on tibia concolorous, tarsi with apex fuscous.

As the margination of vertex is very obsolete this species must be referred to the subgenus *Lygocoris*. It is easily distinguished from the known species by its small size, and the black color of the scutellum and the apex of the head.

*Holotype:* ♂, Chingchengshan, Szechuan, July, 1932, collected by G. Liu, in the author's collection.

**Lygus szechuanensis** sp. nov.

MALE. Body oval, length 4.50 mm., width 2.82 mm., brown-black to black, shining, clothed with flavous simple hairs.

*Head:* inclined, width 1.04 mm., length 0.87 mm.; vertex, width 0.24 mm., margin elevated, ferruginous posteriorly; clypeus moderately prominent, confluent with frons; genae low, gula moderately long. Rostrum, length 2.08 mm., barely reaching apex of hind coxae, ferruginous, apex fuscous, segment I not reaching apex of xyphus.

*Antennae:* inserted near apex of interior margin of eyes; segment I, length 0.56 mm., black, sometimes paler; II, length 1.95 mm., distinctly incrassate at apex, sometimes flavous at base; III, length 0.73 mm., paler at base; IV, length 0.70 mm.; last two segments very slender.

Pronotum, length 0.95 mm., width at base 1.86 mm., at apex behind collar 0.77 mm., remotely and coarsely punctate; calli smooth, confluent anteriorly, base before scutellum truncate, sides straight. Mesoscutum partly covered. Scutellum, length 0.65 mm., width at base 1.08 mm., impunctate, but transversely strigose. Xyphus with lateral margins elevated. Ventral side with ostiolar peritreme and the margin of pleura next to coxae flavous.

*Hemelytra:* coarsely punctate; embolar margins moderately curvate,

length 2.17 mm.; cuneus, length 0.87 mm., width at base 0.78 mm., declivent; membrane and veins fuscous.

*Legs:* femora somewhat incrassate, with a subapical ring flavous, sometimes the ring obsolete; tibia with a sub-basal ring and apical half (apex excepted) flavous, spinules fuscous, destitute of black spots at base; tarsal segment II more than twice as long as I, segment III the longest, apex fuscous.

This species must be referred to the subgenus *Lygus*. It may be easily distinguished by the uniformly black body and the incrassate second antennal segment.

*Holotype:* ♂, Chingchengshan, Szechuan, July, 1932, collected by G. Liu, in the author's collection. Paratype: 4 males, taken with the type; 1 male, Mt. Omei, alt. 4,400 ft., Szechuan (D. C. Graham); 1 male, O-Er. 26 mi. north of Li Fan, Szechuan, alt. 9,000 ft., 1933 (D. C. Graham).

**Lygus szechuanensis var. ruficephalus nov.**

Structurally not differing from typical *szechuanensis*, but head (except clypeus), antennal segment I and base of II, anterior part of pronotum, xyphus, propleura, episternum, rostrum (except apex), anterior coxae, femora, and tibiae, reddish.

*Type:* ♂, O-Er, 26 mi. north of Li Fan, Szechuan, alt. 9,000 ft., 1933, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* ♂ and ♀, same data as type; ♂, near Muping, July, 1929 (D. C. Graham); ♂ and ♀, Chingchengshan, Szechuan, July, 1932 (G. Liu); ♂, Pei Bay, Szechuan, July, 1932 (G. Liu); ♂, Kingfoo Shan, Szechuan, Aug., 1932, (G. Liu); and ♂, Mt. Omei, Szechuan, Sept. 15, 1938 (K. F. Chen).

**Eurystylus luteus sp. nov.**

FEMALE. Body oblong, quite robust, length 6.5 mm., width 2.77 mm., flavous, mottled with fuscous; clothed with pale to golden tomentose pubescence, intermixed with minute simple golden and fuscous hairs.

*Head:* inclining, length 1.08 mm., width 1.17 mm.; vertex, width 0.52 mm., immarginate; clypeus prominent, strongly compressed, discrete with frons, a spot at base of antennae black. Eyes black, hind margin pale, collum behind eyes shining black. Rostrum, length 1.95 mm., barely reaching apex of intermediate coxae, segment I surpassing anterior margin of pronotum, III and IV nigro-fuscous.

*Antennae:* segment I, length 1.12 mm., width 0.34 mm., distinctly compressed, constricted at base, clothed with pale tomentose hairs, intermixed with simple fuscous hairs, ferruginous, spotted with pale; II, length 2.25 mm., incrassate at apex, width at apex 0.25 mm., ferruginous, pale at extreme base, gradually darkened at apex, clothed with minute pale pubescence; III, length 0.73 mm., width 0.11 mm.; IV, length 0.57 mm., width 0.07 mm.; the last two segments dark, with base pale, clothed with minute pale pubescence.

Pronotum, length 1.82 mm., width at base 2.38 mm., at apex 1 mm., lateral margins straight, posterior margin rounded, nearly straight or slightly sinuate before base of scutellum, strongly declivent; two subapical transverse spots and two short longitudinal lines on posterior portion fuscous; collar, thickness 0.22 mm., narrowed at sides. Mesoscutum partly covered. Scutellum, length 1.38 mm., width 1.30 mm. Mesosternum chiefly fuscous at middle. Venter flavous, with a sublateral line on each side and vagina exterior dark.

*Hemelytra:* longly surpassing apex of abdomen; embolar margin nearly straight, length 3.16 mm.; interior apical half of corium, base and apex of cuneus chiefly fuscous; cuneus, length 1.04 mm., width at base 1.04 mm., strongly declivent; membrane transparent, rugulose apically, apical margin fumose, veins and an oblique line behind areole fuscous.

*Legs:* cylindrical, comparatively short, ferruginous, spotted with flavous, tending to uniformly ferruginous to fuscous apically; spinules on tibia ferruginous to fuscous.

Allied to *E. sauteri* Popp. but differs in the larger size and different coloration. Posterior margin of pronotum is not broadly sinuate and more than twice as wide as apex. Second antennal segment is about twice as long as the first and scarcely three times as long as the third.

*Holotype:* ♀, Taipingshien, Anhwei, Oct., 1932, collected by G. Liu, in the author's collection. *Paratype:* 2 ♀ ♀, taken with the type.

**Adelphocoris glaucus** sp. nov.

FEMALE. Body elongate, length 8.14 mm., width 2.64 mm., bluish green, shining, smooth, sparsely clothed with fine sericeous pubescence, intermixed with simple fuscous hairs above and fine pale hairs beneath; color beneath uniformly paler than above.

*Head:* inclining, width 1.08 mm., length 0.9 mm.; vertex, width 0.44 mm., slenderly marginate, with a shallow median longitudinal sulcus; frons obsoletely obliquely strigose; clypeus prominent, bending downward, confluent with frons, facial angle forming a right angle. Rostrum, length 2.80 mm., surpassing apex of hind-coxae, apical two-thirds flavous, apex fuscous, segment I reaching middle of xyphus.

*Antennae:* linear, inserted at apical third of interior margin of eyes; segment I, length 0.87 mm., flavous at extreme apex; segment II, length 2.94 mm., flavous at apical half, tending to fuscous or black on apical fourth; segment III, length 1.38 mm., progressively fuscous; segment IV missing.

Pronotum, length 1.29 mm., width at base 2.16 mm., at apex behind collar 0.87 mm., disk posteriorly finely transversely rugulose, basal margin moderately rounded, lateral margins straight; collar somewhat opaque, as thick as antennal segment II; calli small and distinct. Mesoscutum exposed. Scutellum, length 1.08 mm., width at base 1.08 mm., finely transversely rugulose. Xyphus concave, lateral margins elevated.

*Hemelytra:* embolar margins nearly parallel, length 3.89 mm.;

cuneus, length 1.40 mm., width at base 0.87 mm., cuneal fracture distinct; membrane light fuscous, veins flavous to greenish.

*Legs:* of moderate length, color of coxae slightly pallid; femora somewhat cylindrical, extreme apex sometimes flavous; tibia progressively flavous at apex, spinules ferruginous; tarsi flavous, segment III longest, fuscous at apex, claws ferruginous.

Male more slender than female but similar in color and pubescence.

This species can easily be distinguished from the known species by its solid, uniform bluish green color; size and color suggestive of *Allorhinocoris flavous* Salhb., but the structure of the head is of the Adelphocoris type.

*Holotype:* ♀, Mt. Omei, Szechuan, alt. 11,000 ft., Aug. 19, 1934, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Allotype:* ♂, taken with the type. *Paratype:* male and female, taken with the type; male, Wei Chow (65 mi. N. W. Chehgtu), Szechuan, alt. 5,500-9,000 ft., Aug. 5, 1910; and male, Yachow, Szechuan, alt. 2,200-4,000 ft.; Aug. 25, 1930 (D. C. Graham).

**Phytocoris knighti** sp. nov. (Fig. 3)

MALE. Body elongate, length 7.66 mm., width 2.34 mm., principally fuscous, above conspurcate and varied with green areas; sparsely clothed with sericeous pubescence and intermixed with simple hairs, the latter concolorous with their location.

*Head:* subvertical, width 1.08 mm., length 0.91 mm.; vertex, width 0.30 mm., immarginate, green; frons fuscous, clypeus moderately prominent, blackish, confluent with frons; lorum tumid, genae very low, gula short; eyes large and prominent, coarsely granulate. Rostrum, length 2.86 mm., surpassing apex of hind coxae, ferruginous, darker at apex, paler at the joints; apex of segment I green, barely reaching apex of xyphus.

*Antennae:* linear, inserted just below the middle of interior margin of eyes; segment I, length 1.3 mm., thickest near base, fuscous, mottled with greenish; II, length 2.94 mm., blackish, base and middle widely annulated with greenish white; III and IV missing.

Pronotum, length 1.08 mm., width at base 1.95 mm., at apex behind collar 0.82 mm., black, basal margin narrowly and middle of disk through collar very broadly greenish; base moderately rounded, sides slightly sinuate behind middle; collar as thick as antennal segment I; calli small. Mesoscutum exposed, with sides green and middle fuscous. Scutellum, length 0.95 mm., width at base 1.08 mm., green, a small spot at apical third of lateral margins fuscous. Xyphus green, base fuscous, lateral margins elevated, disk planate. Ventral side uniformly fuscous.

*Hemelytra:* fuscous to black, a series of small spots along embolar margin, a large spot beyond the middle of corium, apex of corium, base, apex, and interior margin of clavus, and basal half of cuneus, greenish; corium, length 3.90 mm., embolar margin straight; cuneus, length 1.3

mm., width at base 0.87 mm., membrane fumate, spotted with fuscous, veins greenish and fuscous.

*Legs:* long, femora fuscous, spotted greenish, pale at middle; tibia fuscous, basal half of hind pair with two broad bands greenish white, spinules ferruginous.

Distinguished from known species by the large and prominent eyes, the narrow vertex, and conspicuous coloration of the body.

*Holotype:* ♂, Mt. Omei, alt. 11,000 ft., Szechuan, Aug., 1934, collected by D. C. Graham, in the U. S. Nat. Mus., Washington, D. C. *Paratype:* males, taken with the type. Named in honor of Dr. H. H. Knight who has been assisting the writer in his studies on Miridae.

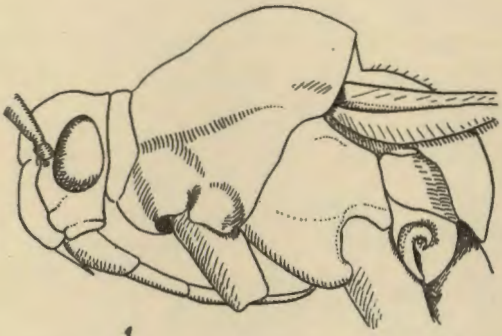
## PLATE I

Fig. 1, *Bryocoris convexicollis* sp. nov.

Fig. 2, *Aretas chinensis* sp. nov., front view of head; a, left genital clasper; b, right genital clasper.

Fig. 3, *Phytocoris knighti* sp. nov.

PLATE I



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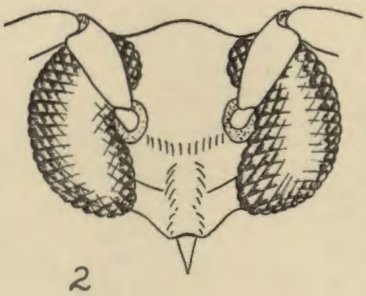

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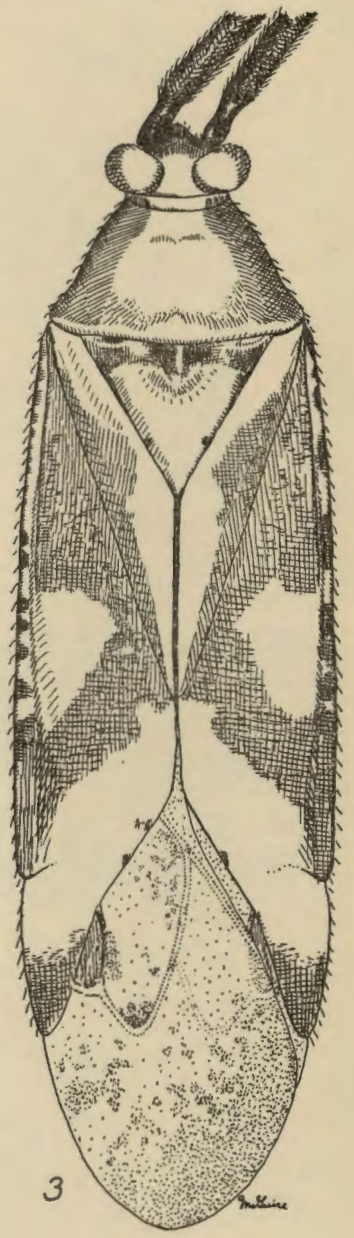

3

# THE DETERMINATION OF URONIC GROUPS IN SOILS AND PLANT MATERIALS

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*From the Soils Subsection, Iowa Agricultural Experiment Station*[2]

Received February 3, 1941

During the past decade it has become apparent that polyuronides and uronic groupings are widely distributed in plant materials, decomposed residues, and soils. In general the uronic content is low, and although determination is a simple matter, the problems to be faced are not the same as when carried out on pure polyuronides, or compounds such as pectin and gums with a high uronic content. The principle of the determination was first elucidated by Lefèvre and Tollens (3), and depends on the measurement of the carbon dioxide evolved on prolonged boiling with 12 per cent hydrochloric acid. Carboxyl groups, other than uronic, occurring in plant materials, do not appear to be decarboxylated under these conditions. The yield of carbon dioxide from pure uronic acids or polyuronides is virtually quantitative. Small quantities of carbon dioxide are evolved, however, from certain non-uronic groups, notably from hexosans. The amount is small, but in materials of high polysaccharide content and low uronic content, the error from these sources is not inappreciable. It is desirable, therefore, that in such cases the determination should be carried out under optimum conditions for the decarboxylation of uronic groups and with a fully standardized procedure that holds constant for any material the error from non-uronic sources.

The apparatus to be described involves no new principles, but the modifications introduced are such that the minimum of attention is required. Inasmuch as the period of determination is ordinarily 4½-5 hours there are obvious advantages in being able to leave the apparatus with complete safety.

## DESCRIPTION OF APPARATUS

The essential parts of the apparatus are similar to those employed by other workers (2) (4). The modifications chiefly concern the methods of control of aeration rate and bath temperature.

The reaction vessel is a 300-ml. pyrex round bottom flask with a standard ground glass joint (24/40) into which is fitted a 30-cm. reflux condenser of the Allihn type (fig. 1). The air intake is led down the center of the condenser and does not dip below the acid in the flask. The air stream is carried to the Truog tower absorbing system by 7-mm. tubing passing enroute through a small trap containing silver sulfate. The tower, about 60 cm. high, is composed of 17 mm. tubing, into which at one end is sealed a small gooch disc, and is filled about two-thirds full of 5-6-mm.

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[2] Journal Paper No. J-840 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 591.

glass beads. To facilitate and to obtain uniformity in washing, a constant delivery pipette (200 ml.) is fixed above the bead towers, filled by gravity from a source of $CO_2$-free water.

Carbon dioxide-free air is drawn through the system by means of a water pump and the rate of flow controlled by capillary tubes. Three capillary controls are provided, one permitting the passage of 1.5-2 l. per hour, another 4-5 l. per hour, and a third, 6-8 l. per hour, with the water pump at full capacity. These controlling capillaries are made by trial from ordinary heavy-walled capillary tubing. One end of each of

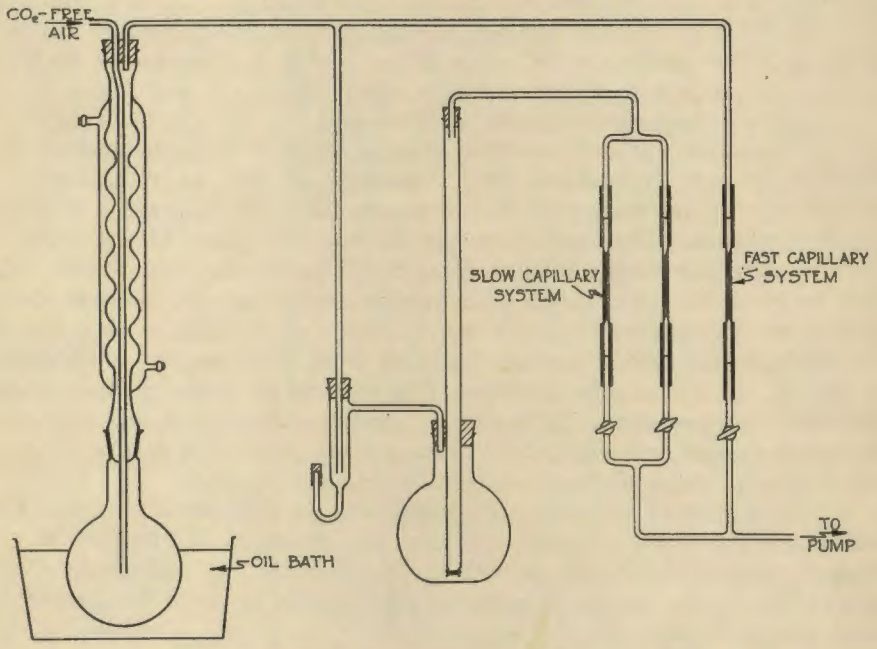


Fig. 1. Diagram of apparatus for the determination of uronic groups in soils and plant materials.

the two slower capillaries is connected through a stop-cock and T-joint to the suction pump guard flask, and the other to the tube leading to the top of the bead towers. The third and fastest capillary is connected to the pump and the aeration system between the condenser and the silver sulfate trap, thus shunting the air stream across the absorbing tower.

The temperature of the reaction flask is maintained by means of an electrically heated oil bath automatically controlled (fig. 2). The thermostat consists of a 100-ml. glass bulb filled with hydrogen sealed to a manometer tube mercury-filled. Two electrodes, one about 12 cm. below the other, are sealed into the arm of the manometer tube. One electrode is to prevent the temperature from exceeding 60° during preliminary aeration, and the other is to maintain the reaction temperature at 135°.

Switches are provided so that one or the other control may be used. When first installed the hydrogen-filled bulb is heated to the desired temperature, and the mercury reservoir attached to the manometer is adjusted for height so that the mercury column makes contact with the lower electrode. The bulb is sealed off so that at 135° the pressure inside equals that of the atmosphere.[3] The heating unit in the bath is connected directly through the thermostat without a relay.

The heating unit is composed of 24 inches of No. 30 nichrome wire wound around two porcelain poles held in a frame of pyrex glass submerged in the oil bath. This controlled heating system has proved very satisfactory in operation.

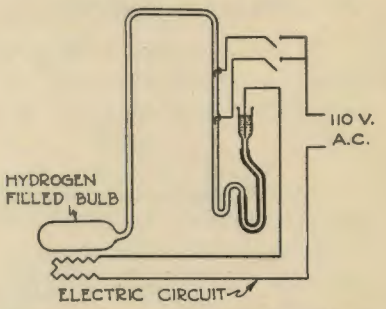


Fig. 2. Diagram of oil bath temperature control system.

## OPERATION

The sample together with 100 ml. 12 per cent HCl are introduced into the boiling flask and aeration commenced through the rapid capillary system, the lower temperature bath heater being switched on at the same time. After 10 minutes the medium or 3-4 l. pr hour capillary control is used, the air stream then being taken through the bead tower. Fifteen minutes later, 25 ml. 0.1 N. NaOH is introduced quickly into the absorbing flask, the slowest capillary system opened, and the high temperature bath control switch closed. The apparatus then requires no attention until the experiment has run its course. Thirty minutes more are required to reach 135°, and aeration and boiling are continued for 4¾ hours at that temperature.

At the conclusion of the run the heating current is shut off, the absorbing flask is lowered and the top of the bead tower opened so that the alkali drains out. The tower is washed down with several aliquots of $CO_2$-free water from the automatic delivery pipette, the same total volume being used in each case. Ten ml. neutral N barium chloride is added and the solution back titrated with standard acid using phenolphthalein. Blank determinations are carried out using 100 ml. 12 per cent HCl only, under precisely the same conditions. The full "operation blank" should not differ from the direct "titration blank" by more than 0.3-0.4 ml. 0.1 N. acid.

## DETERMINATION OF URONIC CONTENT OF SOIL ORGANIC MATTER

The uronic content of soils is quite variable and not necessarily proportionate to the total organic matter present. Ten grams is a suitable amount of soil in most cases, though when the organic content is low it may be necessary to take a larger amount. 100 ml. 12 per cent acid is

[3] Wide fluctuations in atmospheric pressure affect the temperature control and have to be compensated for by slightly raising or lowering the mercury column.

added, and aeration commenced as described above. If the presence of carbonates is suspected, the acid is added several hours before the determination is to be made, and rapid aeration of the suspension begun. The time may be shortened somewhat by heating the flask briefly to a temperature of about 50°, which is insufficient to cause liberation of carbon dioxide from uronic groupings. The carbonate may actually be determined in this operation if desired. No difficulty has been experienced with bumping. The results of duplicate determinations on some soils covering the range ordinarily found are given in Table I.

## DETERMINATION OF URONIC CONTENT OF PLANT MATERIALS

With the exception of certain special cases the uronic content of plant materials is low, and small in proportion to other carbohydrate groupings, such as hexosans, which may yield a little $CO_2$ under the same con-

TABLE I

URONIC CONTENT OF VARIOUS SOILS EXPRESSED AS CARBON DIOXIDE YIELD

| Soil type | No.* | Depth | g. $CO_2$ yield per 100 g. air dry soil |
|---|---|---|---|
| Edina silt loam | P2-1 | 0-4″ | 0.082 ; 0.084 |
| Edina silt loam | P2-6 | 16-19″ | 0.016 ; 0.016 |
| Weller silt loam | P4-1 | 0-1½″ | 0.114 ; 0.118 |
| Weller silt loam | P4-6 | 10-13″ | 0.028 ; 0.030 |
| Ames v. f. sandy loam | 206 | 0-2½″ | 0.232 ; 0.238 |
| Webster silty clay loam | 208 | 0-6″ | 0.162 ; 0.162 |
| Leon fine sand | P213-1 | 0-1½″ | 0.108 ; 0.110 |
| Leon fine sand | P213-2 | 1½-3″ | 0.019 ; 0.021 |

* Numbers refer to soil sample file, Soils Subsection, Iowa Agricultural Experiment Station.

ditions. Perhaps the most difficult situation arises when it is desired to determine with precision the uronic groups present in cellulose preparations, inasmuch as the uronic-derived $CO_2$ may be little if any in excess of that derived from the hexosan. Standardization of experimental conditions and operations are essential to hold constant the yield from the latter source. Whereas uronic groups are quantitatively decarboxylated by 12 per cent HCl in 4½-5 hours, $CO_2$ evolution from hexosans continues apparently indefinitely at a low level. Small variations in the concentration of HCl employed have no effect on the yield of $CO_2$ from uronic units or the time in which quantitative recovery is obtained, but affect considerably the amount of $CO_2$ evolved from hexosan groups, as shown by Whistler *et al.* (6).

The opinion has been expressed that in the case of predominantly hexosan materials, yields of $CO_2$ of the order of 0.2-0.3 per cent in 5 hours cannot be considered to indicate the presence of any uronic groups (5). Campbell, Hirst, and Young (1) in considering the case of starches put the figure higher, and attached no significance to yields of 0.3-0.5 per cent. The yield from a pure monosaccharide such as glucose is not

necessarily identical with that from a polysaccharide. Glucose has been stated to give 0.40 per cent (1), 0.19 per cent (5), and 0.26 per cent (6). Lower figures have been obtained from cellulose preparations believed to be virtually free of uronic groups. Purified cotton cellulose and ramie cellulose have given figures of 0.1 per cent, purified flax cellulose 0.16 per cent (6), hemp cellulose 0.2 per cent (5), and in this work, purified cotton cellulose 0.10 per cent, esparto cellulose 0.16 per cent, acid-hydrolyzed wheat straw cellulose 0.08 per cent, and acid-hydrolyzed chinese hemp cellulose 0.07 per cent. There seems to be clear evidence that the yield of $CO_2$ from glucose is greater than that from cellulose, even though the conditions employed by the various investigators have not been identical. The disturbing effect of the hexosan groups in pure cellulose may therefore have been somewhat over-estimated.

Other hexosans have been little investigated. The yield from monosaccharides other than glucose appears to be considerably higher than from that sugar (1). Fructose undoubtedly yields appreciable amounts of $CO_2$ (*circa* 0.6 per cent) and sucrose also by reason of its fructose component.

Little is known of the behavior of the pentoses and pentose polysaccharides. Both xylose and arabinose have been reported to yield $CO_2$ in excess of that given by glucose (1). The relative reduction in $CO_2$ evolution from xylan-containing and xylan-free celluloses as a result of dilute acid hydrolysis suggests that the xylan fraction of plant celluloses may have a more serious disturbing effect in uronic determinations than the hexosan component.

Uronic determinations on cellulose preparations should desirably be made on samples that have not been dried by heat. Chemically treated samples should invariably be treated wet and a moisture determination carried out separately. Prior knowledge of the moisture content is essential in order that the acid concentration may be adjusted to 12 per cent. The equivalent of 2.5-5 g. dry cellulose should ordinarily be employed, and larger amounts may be desirable if the uronic content is low.

The yields of $CO_2$ from various isolated and treated celluloses are given in Table II. It will be seen that the $CO_2$ yield of many natural celluloses isolated by a standard procedure is quite variable and often considerably above that likely to be obtained from non-uronic sources.

Further evidence of the presence of uronic groups in certain isolated celluloses was obtained in a study of the rate of $CO_2$ evolution during the period of boiling with acid. The absorbing system was changed every 10 minutes during the first hour, every 15 minutes in the second hour and thereafter at thirty-minute intervals. Uronic groups occurring in a polyuronide, such as pectin, are decarboxylated in a characteristic manner, the curve for rate of evolution exhibiting a sharp peak between 20 and 30 minutes from time of boiling. Slightly less than 50 per cent of the final $CO_2$ yield was obtained in the first hour (fig. 3). A similar sharp peak was found in the rate of evolution from fructose and sucrose, the maximum, however, being obtained earlier. Glucose behaves in a

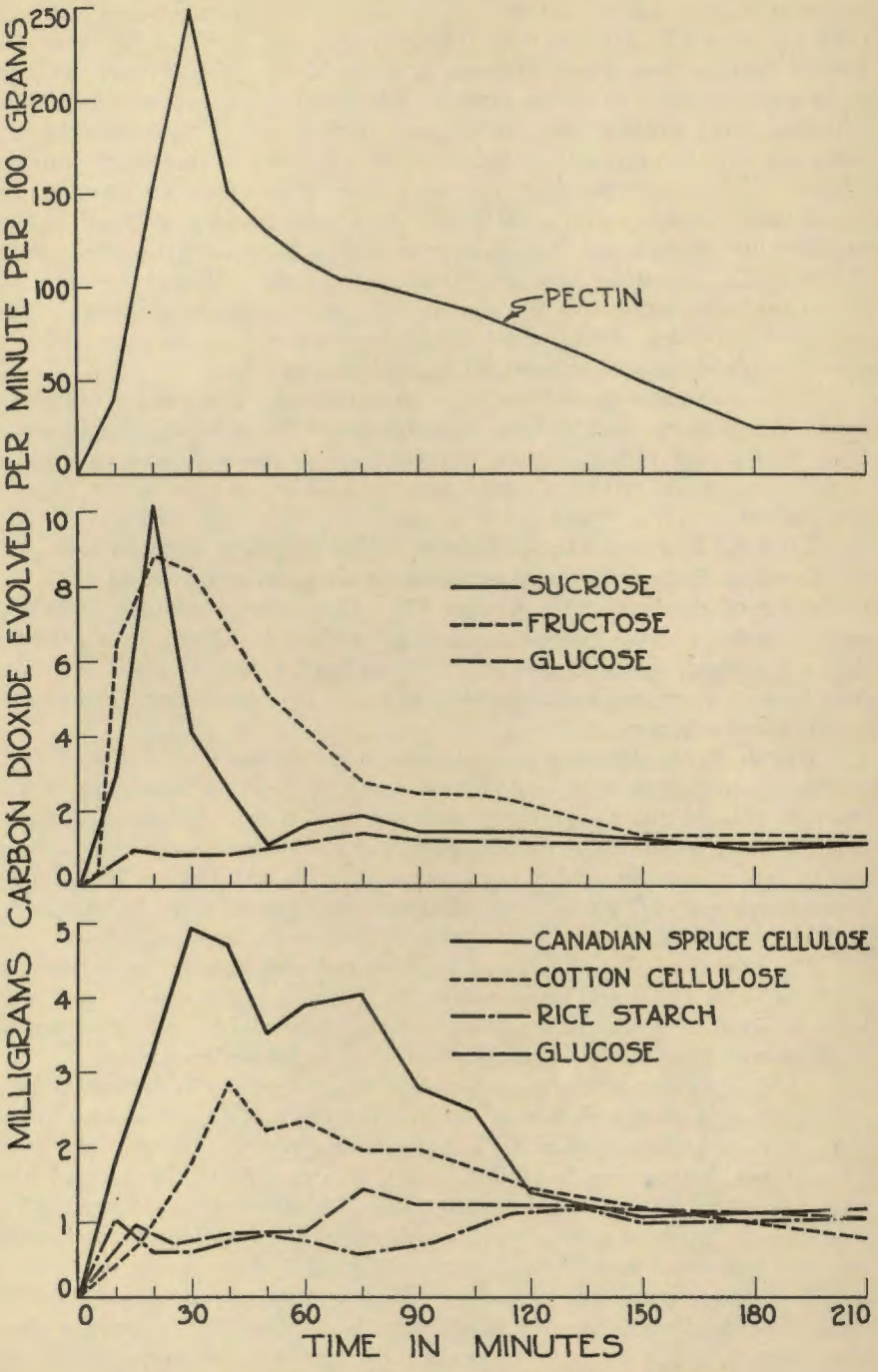


Fig. 3. Rate of evolution of $CO_2$ from various substances.

TABLE II

CARBON DIOXIDE YIELD FROM VARIOUS CELLULOSE PREPARATIONS

Expressed as g. $CO_2$ per 100 g. Oven Dry Cellulose

| Cellulose | $CO_2$ | Cellulose | $CO_2$ |
|---|---|---|---|
| Cotton (raw) | 0.41 | English oak‡ | 0.54 |
| Cotton (purified)* | 0.10 | Canadian spruce‡ | 0.42 |
| " (fermented) | 0.21 | Italian hemp‡ | 0.19 |
| Cotton oxycellulose (hypochlorite) | 0.52 | Silver fir‡ | 0.45 |
| " oxycellulose (hypobromite) | 0.86 | Rye grass (mature)‡ | 0.32 |
| " oxycellulose (dichromate) | 1.52 | Jute‡ | 0.78 |
| Wheat straw‡ | 0.30 | Sisal‡ | 0.82 |
| " " (oven dried) | 0.44 | Esparto | 0.16 |
| " " (acid hydrolyzed)† | 0.08 | Ramie‡ | 0.36 |
| Chinese hemp‡ | 0.37 | Flax‡ | 0.30 |
| " " (acid hydrolyzed)† | 0.07 | Alkali pulp (unbleached) | 0.41 |

* Boiled successively with 1 per cent NaOH and 1 per cent HCl.

† Boiled with 5 per cent sulfuric acid for 1 hour.

‡ Isolated by alternate chlorination in suspension and extraction with hot 3 per cent neutral sodium sulfite until lignin-free.

different manner, evolution being at a slightly higher rate in the second hour than the first. An ill-defined peak seemed to occur at about 75 minutes, and thereafter $CO_2$ was evolved at a steady rate for many hours. The uronic peak occurring in the neighborhood of 20 minutes was detectable in the rate of $CO_2$ evolution from structural celluloses such as that from Canadian spruce (fig. 3) and to a lesser extent also in cotton cellulose. The former exhibited also a smaller maximum at about 75 minutes probably due to hexosan material. No uronic peak was detectable from rice starch.

The figures obtained in rate of $CO_2$ evolution experiments such as these have no absolute value since the $CO_2$ absorbed in short time intervals is in part a characteristic of the size of apparatus in relation to the sample, the rate of aeration, etc. To obtain significant amounts of $CO_2$ in brief intervals the sample size has to be substantially increased over that of normal determinations. In these experiments the amounts employed were the equivalent of 10 g. dry plant cellulose, 18 g. cotton, and 15-20 g. of the sugars and starch, as contrasted with only 1 g. pectin. The general procedure, however, may be used to establish the probable presence or absence of uronic groups in substances giving low yields of $CO_2$, and can be employed to supplement the differential method suggested by Whistler *et al.* (6) which is only applicable if the uronic groups are acid-hydrolyzable and the disturbing hexosan or other groups, acid-resistant.

## SUMMARY

1. The construction and operation of an apparatus for the determination of uronic groups in materials of low uronic content, such as soils

and plant materials, is described. Automatic control of the reaction temperature and aeration rate is provided so that the apparatus once started requires no attention.

2. The determination of uronic groups in cellulose preparations is subject to error caused by $CO_2$ evolution from hexosan groups. The yield from this source, however, is probably lower than that from glucose. Evidence of the presence of true uronic groups in cellulose preparations or similar materials may be obtained by ascertaining the rate of liberation of $CO_2$. A characteristic distribution curve with a sharp peak within the first 30 minutes is given by uronic-containing compounds.

## REFERENCES

1. Campbell, W. G., E. L. Hirst, and G. T. Young
1938. Estimation of uronic anhydride residues in polysaccharides. Nature, 142:912.

2. Dickson, A. D., H. Otterson, and K. P. Link
1930. A method for the determination of uronic acids. J. Amer. Chem. Soc. 52:775-779.

3. Lefevre, K. N., and B. Tollens
1907. Investigations of glucuronic acid, its quantitative determination and its color reaction. Ber. deut. chem. Ges. 40:4513-4523.

4. Nanji, D. R., F. J. Paton, and A. R. Ling
1925. Decarboxylation of polysaccharide acids: its application to the establishment of the constitutions of pectins and to their determination. J. Soc. Chem. Ind. 44:253-258T.

5. Norman, A. G.
1939. Determination of uronic groups in polysaccharides. Nature, 143:284-285.

6. Whistler, R. L., A. R. Martin, and M. Harris
1940. Determination of uronic acids in cellulosic materials. J. Res. Nat. Bur. Stand. 24:13-23.

# THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

## I. GLUCOSE CARBON PARTITION[1]

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*From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station*

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### INTRODUCTION

*Chaetomium funicola* Cke., like other Chaetomium species, was frequently found associated with cellulose materials. This fact suggested an investigation of the possible use of *C. funicola* in commercial utilization of cellulose waste products. Studies were initiated to ascertain (1) the nature and amounts of products produced and accumulated in glucose dissimilation and (2) the mechanism of glucose dissimilation, particularly the nature of the intermediate products formed. The results of a preliminary study pertaining to the first of these points are set forth in the present report and will be followed by further studies.

Literature on the catabolic activity of *C. funicola* was confined to the single work of Lowell (3) who found small amounts of acetic acid to be the only product of the volatile acid fraction when this fungus was cultivated on filter paper suspended in a nutrient salt solution. The formation of other chemical fractions was not reported. Birkinshaw *et al.* (2), using an unidentified *Chaetomium* sp., found that small but definite amounts of nonvolatile acids and nonvolatile neutral compounds and only negligible amounts of volatile neutral compounds were produced from glucose in Czapek-Dox solution. Volatile acids, however, were not formed.

With some modifications in the method, the present study of *C. funicola* was carried out in a manner similar to that followed by Birkinshaw and Raistrick (5) for many different fungi. These modifications comprised (1) determination of a proximate serial carbon balance sheet by the analysis of six cultures of different periods of development, and (2) maintenance of a continuous slow aeration of the cultures in contrast to intermittent aeration.

### MATERIALS AND METHODS

A culture of *Chaetomium funicola* Cke. isolated from baled cornstalks (6) was used. Subcultures of this fungus were prepared on 275 cc. of

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[1] Journal paper No. J-829 of the Iowa Agricultural Experiment Station, Ames, Iowa, Botany and Plant Pathology Section, Project No. 85.

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

[2] Grateful acknowledgement is made to Dr. J. C. Gilman for suggesting the problem and for his constant encouragement and advice throughout its experimental and written stages and to Dr. I. E. Melhus and Dr. C. H. Werkman for their stimulating counsel.

Czapek-Dox liquid medium contained in each of six 2-liter Erlenmeyer flasks. The composition of this medium was as follows: 1 liter distilled water, 50.0 gms. Pfanstiehl's technical glucose, 2.0 gms. $NaNO_3$, 1.0 gm. $KH_2PO_4$, 0.5 gm. KCl, 0.5 gm. $MgSO_4 . 7H_2O$, and 0.01 gm. $FeSO_4 . 7H_2O$. Separate sterilization at 15 lbs. steam pressure for 15 minutes was given to the glucose and mixed salt solutions. The medium in each flask was aseptically seeded with 1 cc. of an aqueous ascospore suspension of the fungus. This suspension was obtained from two-week-old cultures of the fungus growing on the usual potato dextrose agar medium contained in 50 cc. Erlenmeyer flasks.

To provide for the constant aeration of each culture and for the determination of the respiratory carbon dioxide and volatile compounds carried away by the air stream, each 2-liter flask containing the seeded medium was attached to an apparatus assembled for this purpose. A single series of glass containers charged with a 40 per cent solution of KOH, soda lime and a 0.001 per cent solution of $HgCL_2$ rendered the incoming air free of carbon dioxide and micro-organisms. The air was then conducted through a series of connected T-tubes to each of the six culture flasks which were provided with steam sterilized units of glass tubing fixed in two-holed rubber stoppers to serve as inlet and outlet air leads. The air inlet tube extended below the surface of the medium while the outlet tube opened just below the rubber stopper. Nonabsorbent cotton was inserted in the exposed ends of these air leads as further precautionary measure against contaminating organisms. The air stream leaving each culture flask was conducted through a system of units for each flask in which carbon dioxide was determined gravimetrically by absorption in 40 per cent solutions of KOH contained in Bowen potash bulbs, and volatile organic compounds were caught in concentrated $H_2SO_4$. Air was obtained from a centralized compressed air supply and was passed through each culture at a uniformly slow rate of one bubble per second. Phenolphthalein served as an indicator for the renewal of the KOH solutions. The entire apparatus was built about a constant temperature chamber maintained at 28°C. The cultures were placed inside this chamber, whereas the carbon absorption units were placed outside.

For the analysis of a culture of a chosen interval of development, the liquid filtrate was separated from the mycelial mat by decantation at the suction pump through a previously washed, dried, and weighed piece of percale cloth. The mycelial mat was washed several times with hot distilled water and the filtrate was made to 500 cc. volume. The weight of the mycelial mat was obtained by drying at 70°C. for several days followed by 100°C. for one day and maintained in vacuo over concentrated $H_2SO_4$ until weighed. Fractionation of the liquid filtrate into the various carbon fractions was done as follows: A 400-cc. aliquot part of the filtrate was made acid to congo red with dilute $H_2SO_4$ and distilled through a long condenser into an ice-cooled container to obtain 250 cc. of distillate. During this distillation the residual liquid was maintained above a volume of approximately 150 cc. The distillate was then made alkaline to

phenolphthalein with NaOH and further distilled to produce 100 cc. of distillate containing neutral volatile materials. The residues of these two distillations were then combined and tested for acidity to congo red and then subjected to steam distillation to yield the volatile acid fraction in the distillate and the nonvolatile materials in the residue. Two liters of steam distillate were thus obtained. Further subdivision of the nonvolatile residue was made by continuous ether extraction for 48 hours only after the residue had been reduced from a volume of 300 to 400 cc. to approximately 100 cc. with the aid of a vacuum distillation apparatus. With this latter step the excessive frothing was minimized by allowing the nonvolatile residue to enter drop-wise through a long capillary tube into a 1 or 2-liter evacuated distillation flask immersed in a water bath held below 45°C.

Carbon determinations on aliquot parts of the various fractions were made by the method of Osburn and Werkman (9). Carbon dioxide was caught in ascarite and weighed. Care was taken to use only fresh $K_2S_2O_8$ and to maintain good rubber connections in the apparatus. Glucose was determined by the Bertrand modification of the Munson-Walker method. Hydrogen-ion concentration readings were made by the Coleman glass electrode apparatus. Melting points of crystals were obtained with a hot plate fixed on a microscope stage. Correction for thermometer stem exposure (5 inches long) was not applied to the melting point readings.

## EXPERIMENTAL RESULTS

### THE PARTITION OF GLUCOSE CARBON

The carbon balance sheet for the six *C. funicola* cultures is presented in table I. The data show the dissimilation of nearly 55 per cent of the glucose within the test period of 33 days development. The rate of glucose dissimilation was not constant throughout this period but was greater during the later stages. The main product formed in addition to mycelium was carbon dioxide. Assuming 50 per cent carbon in the mycelium, these two products retained 40.0, 73.4, 85.7, 76.9, 68.6, and 79.5 per cent, respectively, of the carbon in the dissimilated glucose for each of the six cultures analyzed at the successive dates. The remaining carbon in part was accounted for by the difference between the total carbon in the medium and the carbon in the residual glucose. The values obtained were 12.9, 36.2, 9.5, 12.1, 23.3, and 15.0 per cent. The difference in carbon in the sum of these corresponding values from 100 per cent remains unaccounted. The figures for initial total carbon were corrected on the basis of 14-cc. loss in volume on sterilization. The assumption that the oven-dried mycelium contained 50 per cent carbon was based on the findings of Birkinshaw *et al.* (1, 2, 3, 4) that in over 200 species of fungi examined, 58 per cent showed a mycelial carbon content of 49-53 per cent and that the species of Chaetomium they examined contained 53 per cent carbon in its dried mycelium (2).

The nature of the nonglucose carbon in the residual medium falls mainly with the nonvolatile, nonacidic, and nonreducing fraction. This

TABLE I

CARBON BALANCE SHEET OF GLUCOSE CARBON PARTITION BY *Chaetomium funicola* CKE.

| | DAYS OF DEVELOPMENT | | | | | |
|---|---|---|---|---|---|---|
| | 3 | 7 | 11 | 18 | 26 | 33 |
| Initial total carbon in the medium, gms. | 5.64 | 5.70 | 5.57 | 5.48 | 5.50 | 5.63 |
| Final total carbon in the medium, gms. | 5.37 | 5.26 | 4.62 | 4.32 | 3.50 | 3.04 |
| Carbon in the medium as glucose, gms. . | 5.33 | 5.01 | 4.52 | 4.16 | 2.88 | 2.58 |
| Carbon in the medium as products other than glucose, gms. ..... | 0.04 | 0.25 | 0.10 | 0.16 | 0.61 | 0.46 |
| Carbon in the medium as neutral volatile compounds, gms. ..... | 0.003 | 0.002 | 0.003 | 0.002 | 0.001 | 0.006 |
| Carbon in the medium as volatile acids, gms. ..... | 0.013 | 0.030 | 0.024 | 0.013 | 0.007 | 0.003 |
| Carbon evolved as respiratory carbon dioxide, gms. ..... | 0.022 | 0.159 | 0.378 | 0.421 | 0.942 | 1.389 |
| pH of medium ..... | 6.2 | 6.4 | 6.8 | 7.1 | 7.7 | 7.1 |
| Weight of mycelium, gms. ..... | 0.204 | 0.693 | 1.045 | 1.189 | 1.711 | 2.102 |

fraction occurred throughout the entire tested period of the experiment and was greatest in amount of formation between the eighteenth and the twenty-sixth day of fungous development when it constituted approximately 35 per cent of the glucose dissimilated in that interval. Relatively abundant early formation of this fraction occurred with the 7-day-old culture. Nonsignificant amounts of nonvolatile acids were formed throughout the tested period. The lack of formation of appreciable amounts of such acids was indicated by (a) the progressive change of pH to the alkaline side, (b) the formation of only small amounts of lead precipitates during the clarification process for sugar analysis, and (c) the formation of only very small amounts of amorphous precipitates as calcium salts insoluble in 80 per cent ethyl alcohol (5). Volatile neutral compound formation was negligible. The volatile acid fraction yielded significant small amounts of carbonaceous material. Volatile acids, however, constituted a very small part of this fraction since at no time was more than 5.0 cc. of 0.02 N NaOH required to titrate this fraction to phenolphthalein. The greater part of the carbon in this fraction was attributed to substances readily carried over by steam distillation. These distillates exhibited very faint cloudiness and a slight paraffin-like scum on the surface.

The percentage recovery of total carbon for the different cultures could not be calculated from the data in Table I because no determinations were made for mycelium carbon. By assuming 50 per cent carbon in the mycelium, however, the percentage recovered total carbon as derived from the carbon in the mycelium, the residual medium, and the evolved carbon dioxide would become 98.4, 101.2, 99.2, 97.3, 96.2, and 97.4 per cent, respectively, for the cultures analyzed at the successive dates. The concentrated $H_2SO_4$ solution in the absorption train of each culture was not

analyzed for the carbon retained because the clear condition of this solution throughout the experimental period was taken as an indication of the absence of any significant amounts of such carbon.

### THE FORMATION OF ORGANIC COMPOUNDS WITHIN THE MEDIUM

Variations in substrate coloration as induced by Chaetomium spp. has been noted by Dickson (7) and Tschudy (10). The nature of the coloring material has not been determined. In the present study *C. funicola* rendered Czapek-Dox solution light yellow during the early stages of its development and brownish-orange in the later stages. Ether extraction of the nonvolatile concentrated residue (made acid or alkaline) yielded a yellowish or a brownish-orange ether solution depending upon the age of the culture and to some extent upon the length of the continuous extraction. The color of the residue was not removed appreciably by this extraction. The ether-soluble fraction contained a reddish-brown fatty liquid and solid materials such as yellow crystalline flakes and short crystalline rods (m.p. 113.0° to 113.5°C.). The fatty liquid was found to have a refractive index of 1.5107 at 24.5°C. and a specific gravity of 0.880 at 21.5°C. Distillation at ordinary pressures and in air produced fumes and an odor of burned fat. A yellow liquid distillate was obtained with a refractive index of 1.5017 at 20°C.

Other substances in the medium were detected in the liquid substrates of nonartifically aerated, 18-day-old *C. funicola* cultures developing on 250 cc. Czapek-Dox medium containing 10 per cent sucrose. The liquid substrates from five such cultures contained in 2-liter Erlenmeyer flasks were bulked, acidified to congo red, and concentrated in vacuo. A solidified mass was obtained, which on extraction with ether yielded approximately 0.5 gm. of a reddish-brown material. Fractionation of this material with solvents yielded the substances as shown in Figure 1. Because of the very small amounts of materials on hand, further purification of the various fractions was not followed. The results, however, indicate the formation of different organic substances in the medium.

### EFFECT OF AERATION AND AGITATION OF THE MEDIUM

Since in the foregoing experiment the culture medium was only slightly agitated by the slow incoming air and the fungus readily developed into a surface mat, an attempt was made to induce the fungus to grow throughout the medium. For this purpose a 2-liter Erlenmeyer flask was used in which were placed 1,500 cc. of Czapek-Dox solution containing 4 per cent glucose. To it were added aseptically 150 cc. of a liquid culture of similar composition contained in a 2-liter flask in which the organism had developed for a period of three days. A vigorous stream of carbon-dioxide-free air was passed through the medium by means of a four-way-outlet glass tube set deep in the medium. The agitation induced by the air stream was sufficient to keep the medium in constantly slow rotatory motion. In the presence of this agitation the fungus developed profusely throughout the medium as small individual colonies that gradually

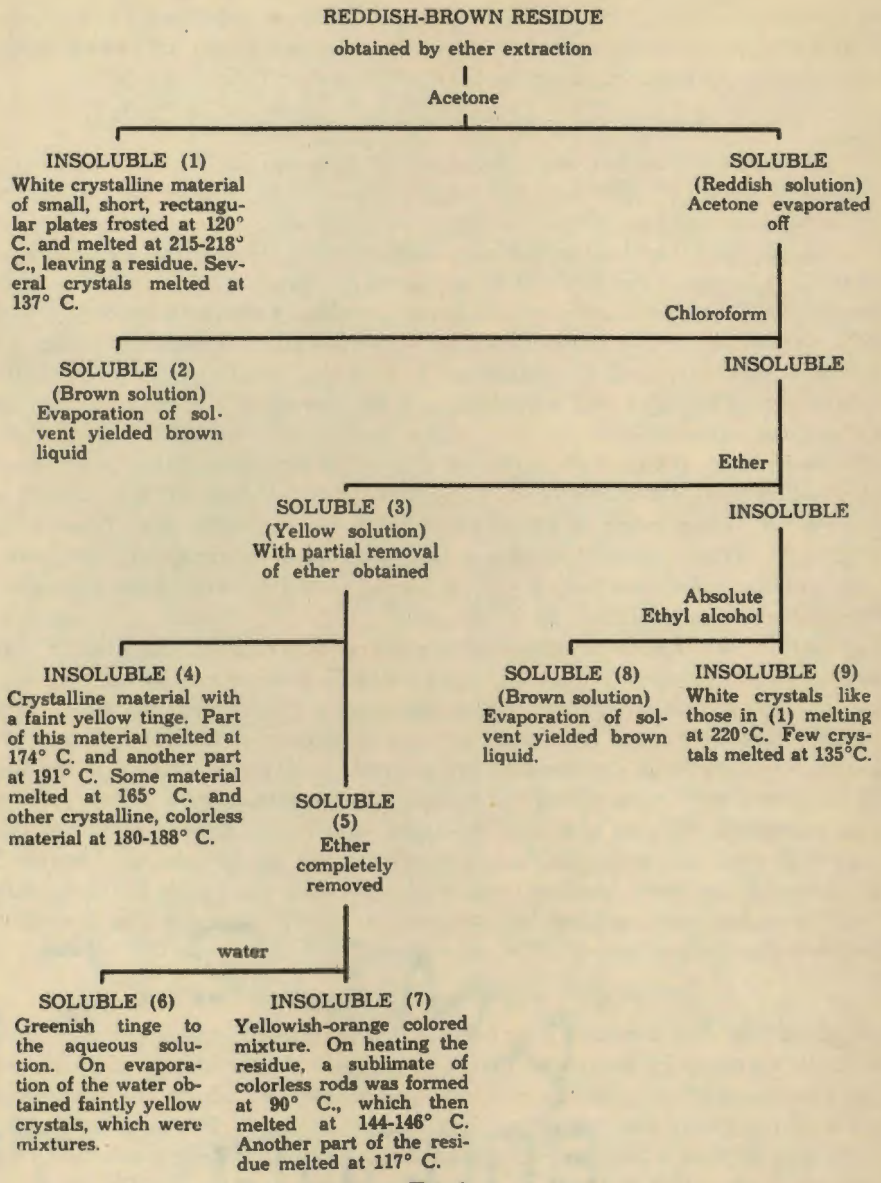


FIG. 1

increased in size. After approximately two weeks of agitation, the colonies increased to a size that made further agitation difficult. At this time the culture became contaminated with bacteria, and the experiment was discontinued. The results for sugar analysis and pH of the medium are presented in Table II.

The data show that even under this condition of vigorous aeration, agitation, and growth of the fungus throughout the medium, the pH of

TABLE II

EFFECT OF AGITATION AND AERATION ON A CULTURE OF *Chaetomium funicola* CKE.

| Time of Agitation and Aeration (days) | pH of the Medium | Milligrams of Glucose Present per 1.0 cc. of Medium |
|---|---|---|
| 0 | 5.01 | 41.75 |
| 1 | 5.75 | 40.20 |
| 5 | 6.55 | 38.30 |
| 17 | 6.30 | 34.10 |

the medium again moved toward neutrality, and at the same time glucose decomposition was still a slow process. Since the air that passed through the medium was in fairly large-sized bubbles, the aeration efficiency probably was not high despite the rapid passage of air through the medium. Attempts to subdivide the air into fine bubbles by passage through small blocks of wood immersed in the medium presented a difficulty as the organism grew into the pores of the wood. Similar difficulty was encountered in the above experiment in using the four-way-outlet glass tubing.

## SUMMARY

*Chaetomium funicola* Cke. slowly converted the glucose in Czapek-Dox solution mainly to carbon dioxide and mycelium over the entire tested period of 33 days development. Products of the nonvolatile, nonacidic, and nonreducing class accumulated in the medium throughout this period. The maximum formation of these products occurred between the eighteenth and the twenty-sixth day of fungous development when approximately 35 per cent of the glucose carbon dissimilated in that period was converted into these products.

Volatile and nonvolatile acids and volatile neutral compounds accumulated in only very small amounts.

The medium gradually became alkaline with progressive development of the fungus.

Ether extraction of the concentrated residual medium yielded a mixture of brownish, liquid fatty material and crystalline organic compounds possessing different melting points.

Vigorous agitation of the culture medium by means of forced air did not hasten glucose dissimilation even though *C. funicola* grew throughout the medium.

## LITERATURE CITED

1. BIRKINSHAW, J. H., J. H. V. CHARLES, A. C. HETHERINGTON, AND H. RAISTRICK
1931. Studies in the biochemistry of micro-organisms. Part IV. Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by species of Penicillium (including Citromyces). Phil. Trans. Roy. Soc. London, Ser. B. 220:55-92.

2. ———
1931. Studies in the biochemistry of micro-organisms. Part VI. Quantitative examination by the the carbon balance sheet method of the types of products formed from glucose by miscellaneous species of fungi. Phil. Trans. Roy. Soc. London, Ser. B. 220: 99-125.

3. BIRKINSHAW, J. H., J. H. V. CHARLES, H. RAISTRICK, AND J. A. R. STOYLE
1931. Studies in the biochemistry of micro-organisms. Part III. Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by species of Aspergillus. Phil. Trans. Roy. Soc. London, Ser. B. 220: 27-54.

4. ———
1931. Studies in the biochemistry of micro-organisms. Part V. Quantitative examination by the carbon balance sheet method of the types of products formed from glucose by species of Fusarium. Phil. Trans. Roy. Soc. London, Ser. B. 220: 93-98.

5. BIRKINSHAW, J. H., AND H. RAISTRICK
1931. Studies in the biochemistry of micro-organsms. Part II. Quantitative methods and technique of investigation of the products of metabolism of micro-organisms. Phil. Trans. Roy. Soc. London, Ser. B. 220: 11-26.

6. BRETZ, T. W.
1931. The fungous flora of baled corn stalks. Master's Thesis, Library, Iowa State College, 110 pp. (Unpublished).

7. DICKSON, H.
1933. Saltation induced by X-rays in seven species of Chaetomium. Ann. Bot. 47: 735-755.

8. LOWELL, C. L.
1931. Industrial biosynthesis of certain organic acids. Doctoral Thesis, Univ. of Iowa. 15 pp. Commercial Press, Lafayette, Indiana.

9. OSBURN, O. L., AND C. H. WERKMAN
1932. Determination of carbon in fermented liquors. Ind. Eng. Chem. Anal. ed. 4: 1-6.

10. TSCHUDY, R. H.
1937. Experimental morphology of some species of Chaetomium. II. Reactions of species of Chaetomium under various conditions of cultivation. Amer. Jour. Bot. 24: 657-665.

# NEW SPECIES OF LYGUS FROM THE WESTERN UNITED STATES (HEMIPTERA, MIRIDAE)

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The writer published a revision of the genus Lygus (1917),[1] but since that time more collecting has revealed a few additional species from the western United States. Van Duzee (1918) described *Lygus abroniae* from California and later *Camptobrochis slevini* Van D. (1925). The latter has proved to be a dark color variety of *Lygus abroniae* Van D. The present paper describes four new species and a variety, some of which have turned up in economic studies by various workers.

### **Lygus ceanothi** new species

Allied to *convexicollis* Reuter but differs in the shorter rostrum, differently shaped head, and coloration of the hemelytra.

MALE. Length 6.7 mm., width 2.8 mm. Head: width 1.14 mm., vertex .41 mm.; position subvertical, frons obsoletely striated, basal carina distinct, a triangular impression just before on vertex. Rostrum, length 2.81 mm., extending to sixth ventral segment, brownish, apex black. Antennae: first segment, length .61 mm., yellowish brown, blackish beneath; II, 1.95 mm., cylindrical, slightly more slender near base, yellowish to reddish brown, narrow base and apical one-third blackish, thickly clothed with short yellowish pubescence; III, .93 mm., black; IV, .61 mm., black.

Pronotum: length 1.27 mm., width at base 2.29 mm., moderately convex, coarsely punctate, shining, a minute yellowish hair arising from each puncture; yellowish brown, calli and two rays behind each callus, traversing disk and joining submarginal area, black; propleura pallid on lower half, a black ray beginning at top of coxal cleft and flaring toward basal margin. Scutellum moderately convex, coarsely punctate, somewhat rugulose; blackish, a median ray on apical half and a short ray each side beginning at base, pallid to yellowish.

Hemelytra elongate, embolar margins only very slightly curved, shining, punctate, clothed with short yellowish pubescence; ground color pallid, subtranslucent, clavus and corium blackish, the dark color broken by pallid spots, the central area of corium more pallid than dark but the apical area is unbroken by spots. Cuneus, length 1.38 mm., width at basal fracture .65 mm., whitish, subopaque, apex and spot at outer basal angle black. Membrane fuscous, basal half of cells, veins, and spot behind tip of cuneus, clear to dusky.

[1] New York (Cornell) Agr. Exp. Sta., Bul. 391, pp. 553-645, 1917.

Legs yellowish to brownish, femora with two subapical bands fuscous to black; tibiae yellowish, base with two dark marks, apex fuscous, spines brownish. Venter yellowish to brownish, sides with a somewhat broken brownish black stripe, more pallid just beneath. Genital claspers very similar to *convexicollis* Reut., but claw at tip of right clasper, obliquely angulate beginning at middle.

FEMALE. Length 6.7 mm., width 3 mm. Head: width 1.25 mm., vertex .52 mm. Antennae: first segment, length .58 mm.; II, 1.77 mm.; III, .90 mm.; IV, .65 mm. Pronotum: length 1.34 mm., width at base 2.55 mm. More robust than the male but very similar in coloration.

HOLOTYPE: ♂ August 20, 1925, alt. 8,500 feet, Pingree Park, Colorado (H. H. Knight); author's collection. ALLOTYPE: same data as the type. PARATYPES: 8 ♂ 8 ♀, taken with the types on *Ceanothus velutinus*. ♀ August 27, 1920, alt. 8,800 ft., Pingree Park, Colorado (H. C. Severin). IDAHO: 11 ♂ ♀, Aug. 6, 1939, Moscow Mountain, alt. 4,000 ft., Moscow (B. C. Fluke). OREGON: ♂ Aug. 7, 4,350 ft.; ♂, Aug. 29, 1930, alt. 6,600 ft., Crater Lake Park (H. A. Scullen).

**Lygus ceanothi delecticus** new variety

Differs from typical *ceanothi* in being less elongate and paler in color; cuneus pallid translucent, without black apex; corium only lightly infuscated apically; pronotum uniformly yellowish to brownish, a small spot behind each inner angle of calli, a larger submarginal spot on humeral angles of disk, and small ray behind top of coxal cleft, black. Scutellum with geminate black mark at middle of base, but the typical pallid marks outlined only with brownish. Femora with brownish subapical rings, base of tibia with black spot.

MALE. Length 5.8 mm., width 2.6 mm. Head: width 1.17 mm., vertex .43 mm. Rostrum, length 2.7 mm., scarcely reaching base of fourth ventral segment. Antennae: first segment, length .69 mm.; II, 2.03 mm., brownish, apical one-fourth blackish; III, .91 mm.; IV, .65 mm. Pronotum: length 1.3 mm., width at base 2.16 mm.

FEMALE. Length 6.5 mm., width 2.9 mm. Head: width 1.17 mm., vertex .47 mm. Antennae: segment I, length .65 mm.; II, 1.95 mm.; III, .95 mm.; IV, .78 mm. Pronotum: length 1.47 mm., width at base 2.47 mm. More robust than the male but very similar in coloration.

HOLOTYPE: ♂ Aug. 6, 1939, alt. 4,000 ft., Moscow Mountain, Moscow, Idaho (B. C. Fluke); author's collection. PARATYPES: 48 ♂ ♀, taken with the type. IDAHO: ♂ 3 ♀ Aug. 4, 1936, Moscow Mountain (Shull & Coon). ♀ Sept. 5, 1932, Moscow Mountain (T. A. Brindley). MONTANA: ♀ July 27, 1918, St. Regis Pass (A. L. Melander). ♂ Aug. 14, 1926, Park County, alt. 6,000 ft. (A. A. Nichol).

**Lygus nigrosignatus** new species

Allied to *elisus* Van D., but antennal segments shorter, body more elongate, and strongly marked with black, head with distinctive marks.

MALE. Length 5.6 mm., width 2.5 mm. Head: width 1.14 mm., vertex

46 mm.; pallid or greenish, a black spot on middle of vertex, often extending as a V-shaped mark on frons; a broad black line extending from tylus across middle of jugum to base of antenna, thence vertically as a narrow line to inner margin of eye, sutural margins of lora and gula also black. Rostrum, length 2.12 mm., almost but not quite attaining posterior margins of hind coxae, greenish, apical segment black. Antennae: segment I, length .45 mm., black beneath, greenish above; II, 1.36 mm., greenish to yellowish brown, narrowly black at base, fuscous to blackish on apex; III, .73 mm., brownish to fuscous; IV, .58 mm., brownish black.

Pronotum: length 1.3 mm., width at base 2.18 mm.; disk somewhat more coarsely punctate than in *elisus*, ground color pallid to yellowish green, inner margins of calli, two black stripes behind each callus, the outer one invading disk of callus, and both stripes extending to join the black sub-basal margin; a small spot at middle of lateral margin of disk and mark behind top of coxal cleft, black. Scutellum pallid, middle of base with broad black mark, bifid at tip and extending to middle of disk, also a more slender black line on each side extending from middle of disk to basal angles; disk coarsely, rugulose punctate.

Hemelytra pallid translucent and marked with black; middle third of clavus except claval vein and corium except for spot on base and on middle, black; apex of embolium and extending upon outer basal angle of cuneus, and apex of cuneus, black; punctate, clothed with short, pallid recumbent pubescence. Membrane clear, veins appear white, a brownish calloused spot next to apical angle of brachium.

Legs pallid to greenish yellow, femora chiefly blackish, a pair of pallid bands before apex, also an incomplete pale band near middle; tibial spines black, knees with two black spots, one below the other; claws and tips of tarsi black. Venter blackish, sides greenish, a black longitudinal line dividing the greenish color.

FEMALE. Length 5.3 mm., width 2.46 mm. Head: width 1.17 mm., vertex .47 mm. Antennae: segment I, length .39 mm., not equal to width of vertex; II, 1.27 mm.; III, .73 mm.; IV, .58 mm. Pronotum: length 1.25 mm., width at base 2.16 mm. Very similar to the male in form and coloration.

HOLOTYPE: ♂ March 24, 1934, alt. 550 ft., Lewiston, Idaho (H. Bergen); author's collection. ALLOTYPE: same data as the type. PARATYPES: 2 ♂, taken with the types on alfalfa. WASHINGTON: 2 ♂ June, 1940, Wenatchee (J. B. Moore); Expt. no. 68, reported injurious to young peach fruits.

**Lygus rolfsi** new species

Distinguished by the elongate form, relatively long antennal segments and the black color markings on a pallid to green ground color.

MALE. Length 6.7 mm., width 2.5 mm. Head: width 1.17 mm., vertex .43 mm.; basal carina strong, sinuate at middle, vertex impressed across base and curving forward on median line; frons with oblique striate lines each side of middle; color yellowish brown, tylus fuscous; gula,

bucculae, margins of lora, basal half of juga, and line extending above base of antenna, black. Rostrum, length 2.2 mm., extending to near apices of hind coxae, brownish to fuscous, apical segment and basal one largely, blackish. Antennae: segment I, length .56 mm., black, brownish to fuscous above; II, 1.82 mm., black, cylindrical, more slender at base, thickly clothed with short yellowish pubescence; III, .82 mm., black; IV, .51 mm., black.

Pronotum: length 1.34 mm., width at base 2.16 mm.; disk moderately convex, coarsely punctate, shining; color pallid to greenish yellow, calli and extending from outer angles to collar, two rays behind each callus and extending to middle of disk, basal submargin of disk and ray extending posteriorly from top of coxal cleft, black. Scutellum greenish yellow, middle of base with broad black ray extending to middle of disk, also an irregular fuscous to black line parallel with lateral margins of disk and nearly joining with the central black ray; coarsely, rugulose punctate; mesoscutum black.

Hemelytra long, embolium 2.68 mm., cuneus length 1.25 mm., width of basal fracture .60 mm.; ground color pallid subtranslucent, tinged yellowish or green, clavus except apical one-fourth, apical area of corium and irregularly bordering clavus, apical area of embolium except outer half, apex and cloud on basal half of cuneus, black. Membrane fuscous, basal half of areoles and area beyond tip of cuneus pale translucent, veins yellowish to reddish. Mesosternum and epipleura largely, black.

Legs fuscous to black, femora with two subapical bands and a broader band at slightly beyond middle, pallid; tibiae yellowish, basal marks and spines black, tarsi and tips of tibiae blackish. Venter pallid to yellowish, ventral surface and lateral line blackish.

HOLOTYPE: ♂ July 15, 1932, Yakima, Washington (A. R. Rolfs); author's collection. PARATYPE: ♂ June, 1928, Los Angeles, California (L. J. Muchmore), collected at light. This species is named in honor of the collector, Mr. A. R. Rolfs, who has favored the writer with a number of western Miridae.

**Lygus shulli** new species

Allied to *hesperus* Kngt., but size smaller and rostrum shorter; size and coloration suggestive of *oblineatus* Say but differs in having only one vertical black line each side of frons; scutellum yellow, with geminate black mark at middle of base; right genital clasper with terminal claw broadly arcuate.

MALE. Length 5.6 mm., width 2.7 mm. Head: width 1.17 mm., vertex .48 mm.; pale yellowish, a vertical black line (frequently obsolete) forming above base of antenna and extending to near top of eye, the apex curved inward on vertex; ventral margin of lora and sometimes a spot on juga, blackish; collum black. Rostrum, length 2.3 mm., extending very slightly behind tips of hind coxae, yellowish brown, apex black. Antennae: segment I, length .56 mm., yellowish brown, blackish beneath; II, 1.73 mm., cylindrical, tapering to more slender on basal half, yellowish brown,

narrow base and apical one-fourth fuscous to blackish; III, .86 mm., blackish; IV, .66 mm., black.

Pronotum: length 1.28 mm., width at base 2.25 mm.; yellowish to brown, outer half of callus and extending forward to sides of collar, two rays behind each callus, the outer one longer and usually extending to middle of disk, a rounded spot on basal angles of disk and sometimes the whole basal submargin, black; propleura with a strong black ray extending from top of coxal cleft to posterior margin. Mesoscutum black. Scutellum pallid to yellowish, a geminate black mark at middle of basal margin, in darkest forms extending to middle of disk; disk coarsely rugulose punctate. Dorsum punctate and shining much as in *oblineatus* Say.

Hemelytra pallid to yellowish brown, subtranslucent, middle of clavus and apical area of corium more or less fuscous to blackish; cuneus pallid, apex and spot on outer basal angle, black. Membrane pale to fumate, apical half except central area and within apices of areoles, fuscous. Legs pale yellowish, femora with a pair of fuscous to black subapical rings, anterior aspect with a series of fuscous spots; tibiae with spot in knee and incomplete ring just below, black, spines also black; tarsi yellowish, spices and claws fuscous. Venter dark brown to fuscous, each side with a longitudinal yellowish stripe. Genital claspers rather similar to those of *oblineatus* Say but the terminal claw of right clasper broadly arcuate, not angulate.

FEMALE. Length 5.2 mm., width 2.8 mm.; costal margin distinctly arcuate in outline. Head: width 1.17 mm., vertex .49 mm. Rostrum, length 2.42 mm. Antennae: first segment, length .57 mm.; II, 1.78 mm.; III, .86 mm.; IV, .66 mm. Pronotum: length 1.3 mm., width at base 2.22 mm. More robust than the male but very similar in color and pubescence.

HOLOTYPE: ♂ July 13, 1936, Mesa, Idaho (W. E. Shull), author's collection. ALLOTYPE: same data as the type. PARATYPE: 78 ♂ ♀, taken with the types. 18 ♂ ♀ July 30, 1932, Belview, Idaho (W. E. Shull), taken on smart weed (*Polygonum* sp.). ♂ 2 ♀, June, 1940, Wenatchee, Washington (J. B. Moore); (Expt. nos. 22, 85, and 99) injurious to young peach fruits.

Named in honor of Dr. W. E. Shull, University of Idaho, who has done important biological work on *Lygus elisus* Van D. and *L. hesperus* Knt., in Idaho.

# NOTES ON THE FAMILY MESOVELIIDAE (HEMIPTERA) WITH DESCRIPTIONS OF TWO NEW SPECIES

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The Mesoveliidae constitute a small family of less than two dozen known species distributed in four genera. The representatives of the family live on the surface of water in secluded coves of marshes, ponds, and lakes and in the wider reaches of streams where the water is more or less quiet and carries an abundance of floating aquatic plants. Some of the species are widely distributed. In the Western Hemisphere *Mesovelia mulsanti* White is the commonest form and occurs generally in North, South, and Insular America. A catalogue of the species then known was published by Horvath in 1929 as Fascicle II of the General Catalogue of the Hemiptera. The types of the new species described below are in the collection of the authors.

### Mesovelia amoena Uhler

*Mesovelia amoena* Uhler, Proc. Zool. Soc. London, 1894, p. 218; Jaczewski, Ann. Mus. Zool. Polonici, IX, 1930, p. 9.

Two winged females from the original type series, H. E. Summer's collection, are at hand. The species appears to have a very wide distribution and to show considerable variation in the arrangement of the light and dark color markings. Apterous and winged females are present from Trinidad, B.W.I., Oct. 27-29, 1938, C. J. Drake; Para, Brazil, H. H. Smith; Canal Zone, Panama, and Barro Colorado Island, February, 1939, C. J. Drake; and Mayaguez, Puerto Rico, June 2, 1938, M. Airlez. Several specimens, the females of which appear to be specifically inseparable from the above-mentioned examples, are present from Laurel, Mississippi, Aug. 27, 1934, H. M. Harris; Baker, Louisiana, August 30, 1934, H. M. Harris; and Waco, Texas, August 9, 1933, H. B. Mills. Males of these are also at hand, but as noted by Jaczewski, male specimens from insular America will have to be studied in order to show the relation of *M. douglasensis* Hungerford and *M. amoena* Uhler.

### Mesovelia bila Jaczewski

*Mesovelia bila* Jaczewski, Ann. Mus. Zool. Polonici, VII, 1928, p. 77, pl. IV, figs. 10-13.

Two males and four females, all apterous, taken in floating aquatic plants in small, stagnant pools in an irrigation ditch about 10 miles from Tigre, Province of Buenos Aires, Argentina, Dec. 10 and 20, 1938, by C. J. Drake. One apterous female, labeled "Tigre, Buenos Aires," from

M. S. Pennington Collection. Known heretofore only from the type locality, State of Parana, Brazil. The alate form is unknown.

This species is smaller than *M. mulsanti* and lacks the spines within on the anterior femora. It is larger than the other known American members of the genus. The parameres, the two tufts of short, black spines near the hind margin of the antepenultimate segment of venter, and the fringe of spines along the posterior margin of the sixth abdominal sternite are distinctive characters.

**Mesovelia zeteki**, sp. nov.

WINGED MALE: Form of *M. bila* Jaczewski, but a little smaller. Pale testaceous, the pronotum and veins of hemelytra considerably infuscated. Head testaceous, with the usual dark, setiferous spots, the interocular space slightly greater than the diameter of an eye. Eyes large, dark reddish brown, coarsely faceted. Rostrum long, reaching on base of venter; testaceous, the apical segment dark. Antennae long, segment I stout, slightly bowed and becoming slender distally, with long bristly hairs at apical third, its length slightly less than width of head through eyes (24:27); segment II a little shorter and slenderer than I; III and IV long, slender.

Pronotum distinctly broader than long (40:26), moderately narrowed anteriorly, its sides and most of posterior lobe dark fuscous, the anterior lobe mostly testaceous, the hind lobe arched, with an indistinct median pale longitudinal line. Scutellum dark, slightly larger and distinctly broader at apex than in *M. amoena* Uhler, its distal portion not so strongly impressed as in that species. Hemelytra with basal portion of clavus and most of corium white, the nervures thickened, dark fuscous. Legs moderately long, testaceous, the front femora unarmed, the long bristly hairs of tibiae and the connexivum margined with brown. Venter pale brown, the three apical segments testaceous; the penultimate segment, broadly angularly produced behind, beset along the margin with dark fuscous bristly hairs. Genital segments plump, rather closely pubescent.

Length (to apex of abdomen), 2.45 mm.; width (through humeri), 0.82 mm.

HOLOTYPE, male, old Panama City, Panama, February 10, 1939, C. J. Drake, taken near the bank in the wide reaches of a small stream. Named in honor of Mr. James Zetek who has done much to increase our knowledge of the insect fauna of the Canal Zone, Panama.

*M. zeteki* lacks the dense tufts of spines on the genital segments which are characteristic of *M. mulsanti* White and its relatives. It is perhaps nearest *M. bila*, however, the irregular row of spines along the margin of the penultimate segment of venter is limited to the angularly produced median portion and does not extend all the way across the section as in *M. bila*, and the tufts of spines are also not present on the

apical segment. The winged male is distinctly larger than the winged female of *M. amoena* Uhler.

**Mesovelia hackeri, sp. nov.**

MACROPTEROUS MALE: Shorter and distinctly slenderer than *M. hungerfordi* Hale, the genital segments without dense tufts of spines. Dark brown, the front lobe of pronotum and head yellowish brown. Head with the usual dark setiferous spots, a narrow median, double stripe paler. Interocular space faintly greater than diameter of one eye. Rostrum reaching on base of venter, segment II very long. Antennae dark brown, segment I long, rather slender, slightly bowed; II slenderer and shorter than I; III and IV very slender—proportions, I:II:III:IV= 30:22:46:47.

Pronotum moderately narrowed anteriorly, the posterior lobe arched, impressed within humeral angles. Scutellum broadly rounded apically, the disc of hind lobe impressed, its margins raised. Wings dark brown, the base of clavus paler; veins raised, very dark. Venter narrowed posteriorly, clothed with fine hairs. Genital segments plump. Legs long, slender, testaceous, the fore femora without spines; the tibiae and tarsi slightly darker in color.

LENGTH (to tip of abdomen), 2.65 mm. WIDTH (through humeri), 0.90 mm.

HOLOTYPE: male, Asharove, Australia, Feb. 3, 1931, collected by Henry Hacker, in whose honor the insect is named.

*M. hackeri* is the second species of the genus known from Australia. It has a distinctly shorter head than has *M. ujhelyii* Lundblad from Oceania with which it agrees in the absence of tufts of spines on the genital segment, and the setiferous points on the head are less widely separated. It is not easily confused with *M. hungerfordi* Hale, a common water-strider in Australia.

MESOVELOIDEA WILLIAMSI Hungerford

*Mesoveloidea williamsi* Hungerford, Bul. Brooklyn Ent. Soc., 24: 1929, p. 289; Jaczewski, Proc. Ent. Soc. Wash., 33: 1931, p. 64; Hungerford, Bul. Brooklyn Ent. Soc., 33: 1938, p. 218.

This interesting species was described from Mera, Ecuador, and has since been recorded from Cacholi, Ecuador, and from Costa Rica and Peru. Specimens are at hand from Los Amates, Guatemala, collected by Kellerman, and from Trinidad, B.W.I., Oct. 27, 1938, C. J. Drake, thus further extending the known range of the species. Only the macropterous form has been taken.

# METHODS AND COMPUTATION IN FECAL ANALYSIS WITH REFERENCE TO THE RED FOX[1]

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Fecal analysis is adapted to studies of the feeding tendencies of some mammals. The technique is particularly useful because the many feces obtainable make possible a continuous determination of the relative quantities of foods consumed by animals in limited areas.

It may be worth while to consider those phases of the technique that seem to be weaknesses. Identification of scats and estimation of their age are problems. In most cases, however, knowledge of "sign" and acquaintance with the ecology of the locality reduce the possibilities of error. The results obtained with respect to easily digested foods or those lacking indigestible elements are uncertain, and in some instances the utility of the technique may depend on whether the animal studied consumes significant proportions of such foods. Analysts regularly identify items considered little resistant to digestion; nevertheless, some of these foods doubtless remain undetected. It appears unlikely, however, that these defects can influence the results sufficiently to obscure the principal feeding trends. Identifying foods as carrion in fecal passages is impracticable, but possibly the value attached to carrion determinations has been unduly emphasized. Such identifications are usually offered as evidence supporting a division of foods into prey killed by the feeding animal and that found dead. This segregation is desirable, but too often the evidence on which it is based is fallible. During freezing weather dead animals may remain in a good state of preservation for prolonged periods, and even under milder conditions carcasses are usually found and eaten before they have aged enough to be recognized as carrion. Further, prey may be killed by a predator and later eaten as carrion by the same individual or species.

Investigators are largely agreed in recognizing the value of fecal analysis to continuous food-habit research on biologically known areas; however, there is some disparity of opinion in respect to methods and records. In current research into the food coactions of the northern plains red fox (*Vulpes regalis*) these differences were considered, and it

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[2] Grateful acknowledgement is made of technical assistance by Robert Moorman and Charles Yocom.

was deemed advisable to appraise the relative values of various methods and computations used in fecal analysis.

## EXPERIMENTAL PROCEDURE

Through laboratory experiments it is possible to test the merits of scat analysis as a method of determining food habits. By feeding captive animals known kinds and quantities of food and subsequently collecting and examining their feces it is possible to test and improve methods of analysis and computation.

For the experiments here reported three pens and kennels similar to those used on silver fox farms were obtained in which to confine wild red foxes. Two pens (I and II) contained one fox each and the third pen (III) housed two.

Foods of known weights and kinds were given to the foxes each afternoon between five and six o'clock. Fecal passages were collected regularly each morning between eight and nine o'clock and again at feeding time. Each passage was placed in a separate bag and catalogued. Thirty-six scats were collected in Pen I, thirty-eight in Pen II, and forty-one in Pen III.

In general, the method of preparing the fecal material for analysis was as follows: Each scat was softened in warm water. The parts of diagnostic significance were separated and cleaned by washing the softened material in a sieve (twelve meshes to the inch) over a glass jar; thus, parts that occasionally passed through the sieve were caught. The remaining material was soaked in a glass beaker filled with clear water in which the heavy objects settled out of those that would float. The material was then poured portion by portion into petrie dishes where it was mechanically separated and, excepting the hair, removed with a forceps to a paper blotting towel. During this separation it was found helpful to shift the petrie dish from black to white backgrounds to make different items visible. The hair left in the dish was finally caught in the sieve and removed to the blotting towel. Identifications were based on the diagnostic parts made available by this sorting process.

## METHODS OF ANALYSIS AND COMPUTATION

The results of the analyses were variously computed as recorded in Table I. The figures shown in the table for pen I (*see* footnote 1) might have been of better quality had something been fed to bring the chicken of the last feedings through the digestive tract in normal progression. The figures, however, are accurate enough for the purposes intended. The data are discussed according to the methods of analysis used.

NUMERICAL RECORD OF INDIVIDUALS. The count of individuals was obtained from enumeration of certain predesignated parts as they appeared in the food residuum.

This is not a very satisfactory way, as in nature the predator does not always consume a complete individual in one or even in several feed-

ings. On occasion the red fox takes only a part of animals even so small as mice. The size, desirability, condition, and availability of food have a bearing on the quantity eaten as no doubt has also the extent of competition with other species for the same food.

FREQUENCY OF OCCURRENCE. Evaluation of frequency was based on the number of times the food items occurred in the series of scats examined. The two principal computations applied were determination of percentages of the passages containing a certain food and percentages of the number of occurrences. The latter was obtained through two approaches: (1) Percentages of food items calculated by groups; and (2) percentages figured for all items represented without regard to groups. These computations are self-explanatory excepting the treatment of the food items by groups. In this the food items are arranged in such a group "break-down" as to insure that among those selected for contrast none shall possess greater opportunity for scoring occurrences in a single passage than any other. It is usually thought that occurrences of foods in fecal passages are disproportionately influenced because some foods possess greater quantities of nondigestible parts than others. Chicken is frequently suggested as a food with less opportunity for scoring occurrences because of a proportionately smaller quantity of nondigestible parts. Although the proportion of chicken to rodents eaten by the foxes in the three pens varied greatly in these experiments, the figures obtained by dividing the total weight consumed by the number of passages produced indicate that the quantity of food, irrespective of nondigestible parts, required to produce a passage was nearly constant. In pen I, 66.6 ounces of food were consumed, and 36 passages were produced, an average of 1.85 ounces to the scat. For reasons already mentioned, the figures from pen I are not especially reliable. The averages for pens II and III were 2.321 and 2.434 ounces, respectively. In general, then, the available data indicate that fecal passages are produced approximately in direct proportion to the quantity of food consumed. As many recognizable foods are taken in quantities less than those required to produce a passage, however, allowance must be made through treatment of the food items in groups. For example, a red fox might eat equal quantities of chicken and mouse, the latter representing three species. As the two food groups are equal in quantity, they may be expected to occur in approximately the same number of passages, and that their frequency of appearance would then be accurate evidence of the relative quantities eaten. Should the foods be considered in the light of individual items, however, it then becomes possible to score as many as three occurrences (one for each species) to a single passage for mouse, whereas it would never be possible to score more than one occurrence to the passage for chicken. Computations without regard to grouping, then result in underemphasis of food items with limited scoring powers and, conversely, in overemphasis of items with greater capacities for scoring, as may be seen

TABLE I

| Kind of Food | Food Ingested | | | | Frequency of Occurrence | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Individuals | Weight | Total Weight (Groups) | Total Weight (Items by Group) | Individuals | Occurrences | Total Occurrences (Groups) | Total Occurrences (Items by Group) | Total Occurrences (All Items) | Passages Containing the Food |
| | *No.* | *Ounces* | *Pctg.* | *Pctg.* | *No.* | *No.* | *Pctg.* | *Pctg.* | *Pctg.* | *Pctg.* |
| AVES: *Gallus gallus** | § | 47.00 | 70.57 | 70.57 | | 30 | 62.50 | 62.50 | 48.39 | 83.33 |
| | | 14.90 | 28.93 | 28.93 | | 15 | 30.00 | 30.00 | 20.83 | 39.47 |
| | | 22.00 | 36.15 | 36.15 | | 22 | 37.93 | 37.93 | 31.43 | 53.66 |
| RODENTIA: ..... | 25 | 19.60 | 29.43 | | 13 | 18 | 37.50 | | | 50.00 |
| | 44 | 36.65 | 71.07 | | 22 | 35 | 70.00 | | | 92.10 |
| | 52 | 38.85 | 63.85 | | 10 | 28 | 62.07 | | | 68.29 |
| *Microtus* sp. ..... | 13 | 12.95 | | 19.45 | 8 | 18 | | 21.09 | 29.03 | 50.00 |
| | 21 | 24.00 | | 46.54 | 13 | 34 | | 41.75 | 47.22 | 89.47 |
| | 26 | 25.50 | | 41.91 | 4 | 25 | | 32.33 | 35.71 | 60.98 |
| *Peromyscus* sp. ..... | 10 | 5.85 | | 8.78 | 5 | 12 | | 14.06 | 19.35 | 33.33 |
| | 22 | 11.95 | | 23.17 | 8 | 21 | | 25.79 | 29.17 | 55.26 |
| | 23 | 11.90 | | 19.56 | 5 | 13 | | 16.81 | 18.57 | 31.71 |
| *Mus musculus* ..... | 2 | .80 | | 1.20 | | 2 | | 2.35 | 3.23 | 5.55 |
| | 1 | .70 | | 1.36 | 1 | 2 | | 2.46 | 2.77 | 5.26 |
| | 2 | 1.15 | | 1.89 | 1 | 2 | | 2.59 | 2.86 | 4.88 |
| *Reithrodontomys megalotis*† ..... | 1 | .30 | | .49 | | | | | | |
| Rodentia, undetermined‡ ... | | | | | | 8 | | 10.34 | 11.43 | 19.51 |

* The data from pen I are given for each item in the top line; those from pen II in the middle line; and those from pen III in the bottom line.

† Remains of this young harvest mouse were not identified in the residuum.

in Table I. The desirability of group treatment is further emphasized by the impossibility of identifying by species all remains in fecal passages. This is particularly true of avian remains.

The primary food groups in these experiments were chicken and rodent, and the basic percentages were calculated from a sampling of these groups. Of Aves, chicken was the only representative; hence, further reckoning was not required. It was necessary, however, to calculate percentages for the component items in the rodent portion. Then these percentages were individually multiplied by the percentage ob-

| Analyses of Fecal Passages | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry Weight Method | | | | | | | | Volumetric Method | | | |
| Weights of the Food Remains | Total Weight of the Food Remains (Groups) | Total Weight of the Food Remains (Items by Group) | Total Weight of the Food Remains (All Items) | Average Weight to the Containing Passage | Average Weight to the Containing Passage (Groups) | Average Weight to the Containing Passage (Items by Group) | Average Weight to the Containing Passage (All Items) | Measured Volume of the Food Remains | Total Volume of the Food Remains | Average Volume to the Containing Passage | Total Average Volume to the Containing Passage |
| *Grams* | *Pctg.* | *Pctg.* | *Pctg.* | *Grams* | *Pctg.* | *Pctg.* | *Pctg.* | *c.c.* | *Pctg.* | *c.c.* | *Pctg.* |
| 12.992 | 50.96 | 50.96 | 50.96 | 0.4331 | 38.40 | 38.40 | 35.29 | 27.2 | 43.17 | 0.9066 | 68.69 |
| 1.554 | 7.74 | 7.74 | 7.74 | .1036 | 16.36 | 16.36 | 9.28 | 6.8 | 9.78 | .4533 | 20.19 |
| 2.444 | 11.81 | 11.81 | 11.81 | .1111 | 14.57 | 14.57 | 11.98 | 4.2 | 7.82 | .1024 | 5.47 |
| 12.504 | 49.04 | | | .6946 | 61.60 | | | 35.8 | 56.83 | 1.9888 | 31.31 |
| 18.533 | 92.26 | | | .5295 | 83.64 | | | 62.7 | 90.22 | 1.7914 | 79.81 |
| 18.243 | 88.19 | | | .6515 | 85.43 | | | 49.5 | 92.18 | 1.7696 | 94.53 |
| 10.067 | | 39.48 | 39.48 | .5592 | | 43.38 | 45.57 | | | | |
| 12.810 | | 63.77 | 63.77 | .3768 | | 31.13 | 33.77 | | | | |
| 10.702 | | 69.51 | 69.51 | .4281 | | 44.78 | 46.14 | | | | |
| 2.360 | | 9.26 | 9.26 | .1966 | | 15.25 | 16.02 | | | | |
| 4.921 | | 24.50 | 24.50 | .2343 | | 19.36 | 20.99 | | | | |
| 2.481 | | 16.10 | 16.10 | .1908 | | 19.96 | 20.56 | | | | |
| .077 | | .30 | .30 | .0383 | | 2.97 | 3.12 | | | | |
| .802 | | 3.99 | 3.99 | .4011 | | 33.15 | 35.92 | | | | |
| .396 | | 2.58 | 2.58 | .1978 | | 20.69 | 21.32 | | | | |
| 4.664 | | | | | | | | | | | |

‡ Undetermined rodent evaluations indicate the possible error in the frequency of occurrence calculation.

§ A record of the number of individuals was not practicable here as the foxes would eat only a part of a chicken at a feeding.

tained for their group, thus, providing for a fair interpretation of the relative importance of each item within the group.

Another computation suggests the possibility of developing conversion factors for each food item. The figures obtained by dividing the number of occurrences into the weight fed appear to offer promise. In pen I, 47 ounces of chicken were fed; 30 passages contained chicken. This corresponds to 1.57 ounces to the occurrence. Similar calculations for pens II and III yield 1.00 and 0.99 ounces, respectively. As previously mentioned, the results in pen I, especially for chicken, were somewhat

atypical, as chicken was the last food eaten and no food was fed to bring it through the digestive tract in normal progression. Factors for all rodents are: 1.09, 1.05, and 1.39. Those for the individual genera are: *Microtus* 0.72, 0.71, and 1.02; *Peromyscus* 0.49, 0.57, and 0.92. The obvious increase in the figures calculated for the genera from pen III is of uncertain value because of the undetermined rodent food included. In general, the variation from the mean for these factors is not so great as to rule out the possibilities of their application. Tables of conversion factors for foods likely to be eaten might be prepared for each predator investigated from data gathered in extensive feeding experiments.

DRY WEIGHT. The computations applied to the dry-weight method present two approaches: Percental evaluation of the respective weights of item remains and percental calculation of the average weights of remains of each item to the containing passage.

The diagnostic parts of each passage were allowed to dry at room temperature, and were then separately weighed on an analytic balance. Segregations, as between the mixed remains of different species of mouse in the same passage, were based on percentages of the relative weights of the identifiable bones of each species, the unidentifiable bones and hair being proportioned according to those percentages.

VOLUMETRIC METHOD. Computations were based on volumetric measurements and reckoned in percentages of the total measured volume and also in percentages of the average measured volume to the containing passage. The figures were obtained by measurement of the separated material in cubic centimeters. Measurements were made in a graduated cylinder, and the material was firmly pressed down for each reading.

## INTERPRETATION OF THE NUMBER OF INDIVIDUALS

Interpretations of relative numbers of individuals represented in the analysis may be attempted in predation studies on areas where prey populations have been estimated. In pen I, *Microtus* occurred in 18 passages; 13 individuals had been fed. A single occurrence then corresponded with 0.72 available individuals. Similar figures calculated from the table are: *Microtus* 0.62 and 1.04; *Peromyscus* 0.83, 1.05, and 1.77. The figures as obtained from pen III are probably overemphasized by the presence of undetermined rodent. With more extensive data it would seem possible to establish factors for estimating from the results of fecal analysis the relative numbers of individuals of prey species eaten.

## DISCUSSION

It is granted that objection-proof conclusions with respect to methods of fecal analysis and computation cannot be drawn from these experiments. The information does indicate, however, the desirability of improving laboratory techniques in food-habits analysis and computa-

tion and offers suggestions as to directions in which improvement may be made.

The data, though admittedly limited, leave little doubt as to the interpretative superiority of the frequency of occurrence method of fecal analysis as contrasted with the other methods used in these experiments. The proposal for improvement of this method by computing the relative quantities through grouping of food items may be questioned; however, it may be suggested that any method of calculation that disproportions one group by subdividing another or others, as does the computation usually employed, at least merits suspicion.

Of the computations attempted with the dry-weight method, the percental evaluation of the respective weights of item remains proved most reliable. It is of considerable advantage if the laboratory method used permits keeping abreast of seasonal and unheralded feeding trends, but dry weighing was the most time-consuming and expensive method tested and cannot be recommended.

Percentages calculated from the average volume to the containing passage appeared to provide the most reliable interpretation of the volumetric method. The results are somewhat uncertain as evidenced by the results for pen III. Though not so exacting as the weight method, it demands considerable time and energy. It also limits measurement to the major food groups and thus provides no interpretation of the relative quantities of lesser items.

## SUMMARY

Foods of known weights and kinds were fed to red foxes for the purpose of testing methods of analysis and computation as applied to fecal material in food-habits research.

The following methods of analysis with various computations were tested: (1) Numerical record of individuals; (2) frequency of occurrence; (3) dry weight; and (4) volumetric. The frequency of occurrence method was shown to provide for the most reliable interpretation of the relative quantities of foods consumed. A proposal was made for improvement of the results in the frequency of occurrence analysis by group treatment of the food items identified in such sequence that no group or item should possess greater potential for scoring occurrences in the same passage than those with which it is contrasted. The possibility of developing conversion factors for each food group or item for application to frequency indices in determining relative amounts was suggested. A technique for calculating the relative numbers of individuals represented in the frequency of occurrence analysis was discussed.

# THE USE OF IOWA CLAYS[1] FOR THE CLARIFICATION OF SORGO SIRUP[2]

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## INTRODUCTION

The production of sorgo sirup in the United States decreased from a high of 49½ million gallons annually in 1920 to a low of 9 million gallons in 1930. Since that time an average of about 14 million gallons has been made per year (16). Although the sirup is not used as universally as formerly, it still enjoys a considerable demand as a table sirup. It has a mild acid flavor that is characteristic and unique.

The making of sorgo sirup has in the past been more of an art than a science. Nearly every community at one time had its sirup maker, as evidenced by the amount of equipment still available on many farms. For some time farm production of sirup has declined and factory production has increased. Recent trends toward diversified farming, however, and reductions in surplus crops acreage, have turned farmers' attention to the growing of small amounts of other crops which may be used to reduce living expenses or as cash crops. Sorgo, although classed as a soil-depleting crop, satisfies both of these requirements in that the sirup may be used by the farmer or sold. A yield of about 100 gallons of sirup per acre may be expected in addition to 20 to 30 bushels of seed.

The farmer who wishes to make sorgo sirup encounters two difficulties. First, he must resurrect what is, in many places, almost a forgotten art, and secondly, his product must be sold to a critical buying public that has become accustomed to the clear, light-colored corn and maple table sirups and to the clear sorgo sirup now being produced on a factory scale. The heavy, dark-brown, viscous sirup often made is viewed with suspicion.

The object of the present study was to ascertain the essential details for the production by the Iowa farmer of a clear, high-grade, commercially acceptable sirup which could successfully compete with factory-made products.

It was found that the use of certain types of clay for clarification of sorgo juice resulted in a very fine grade of sirup. Several clays were examined to determine the characteristics necessary for this purpose and to ascertain the optimum conditions for preparing sirup on a farm scale

[1] The word "clay" is used in a general sense to include materials also known as loess and gumbotil.

[2] Journal Paper No. J-846. Iowa Agricultural Experiment Station. Project No. 584.

by this method. Under the same conditions when the older method was compared with the clay clarification method, it was found that the latter method was economically feasible. Although the loss when using clay, was a little greater, the grade of sirup was much improved, and the capacity of the mill was approximately doubled.

## METHODS OF PRODUCING SORGO SIRUPS

The cultivation, growing, harvesting, and milling of sorgo cane are not within the scope of this paper. Briefly, the cane is grown and cultivated about like corn and harvested in September before freezing weather. Leaves are stripped from the cane, and the seed head and two or three top joints are removed (to lessen acidity (15) ). The cane is cut and brought to the cane mill where it is pressed between rollers and the sorgo juice, after being strained through a coarse screen, is then ready to be made into sirup. It should be noted that juice which stands longer than a few hours is very likely to spoil by fermentation, a fact which greatly increases the difficulty of research. A poor tasting sirup may result from cane which is raised on land highly fertilized by manure. Occasionally a red juice is obtained which makes a poor grade of blood-red sirup. The cause is not known but further work on this subject is being carried on at Iowa State College.

The following four general methods of preparing the sirup are used:

1. Factory process: The sorgo juice is clarified by absorbents and heat, and filtered through filter presses. A complete description of factory practice has been given by Bartling (1). The process is not suitable for farm use. The sirup is clear and dark yellow in color with a mild taste since the acidity is controlled. The sirup is generally thinner than farm sorgo sirup and thus can be sold for a lower price.

2. Old process: The farm method used for many years is to coagulate the green material in the juice by boiling and then skimming it off constantly. The process is tedious and laborious, and the product is usually dark brown due partly to the long-continued heating (18).

3. U.S.D.A. process: The method recommended by the United States Department of Agriculture as described in Farmers' Bulletin No. 1791 (16) is essentially an elaboration of the old process mentioned above. For juices that contain starch, which will cause the sirup to "jelly," it is recommended that the juice be partially evaporated and then malt extract added to the semi-sirup to convert the starch. The mixture is allowed to stand over night and the evaporation continued.

4. Clay clarification process: This process involves the addition of a small amount of clay to the cold juice which causes the green material to coagulate. The clarified juice is then decanted from the clay and may be boiled down rapidly with a minimum of skimming. It has been found that this process is being used successfully on some farms and that it gives the best sirup of any examined, including the factory-made pro-

duct. This process was, therefore, studied at considerable length with the object of determining the details of the clay clarification method, comparing its yield with that of the older process on an economic basis, and of examining the clay varieties which gave satisfactory clarification. A preliminary popular report of some observations on this subject has been published by the authors (8).

## HISTORICAL AND THEORETICAL

The use of clay for clarification of sorgo juice is rare but not new. Some writers (3) disparage the use of clay for this purpose. In 1893 the United States Department of Agriculture (17) described the examination of 200 clays for this use and stated that clay is very good for this purpose. Theoretically clay should serve very well as a coagulant. The impurities present in the juice are probably nitrogenous and carbohydrate materials such as chlorophyl, starch etc., all in colloidal suspension. The reaction of the juice is slightly acid. The investigations of Demolon and Barbier (5) show that humic colloids are strongly adsorbed by clay when the solution is acid. Mattson (10) found that the isoelectric point of proteins was lowered by the addition of bentonite, perhaps due to the formation of a non-ionized complex with the proteins. Meyers, too, has observed (12) that the tendency of organic colloids to combine with inorganic soil colloids was greater in acid suspensions and suggested polar adsorption as the probable reaction involved. Ensminger and Giesking (6) have observed that proteins are more strongly adsorbed in suspensions of high hydrogen-ion concentrations than in suspensions of low hydrogen-ion concentrations. They suggest that the adsorption of proteins as cations is partly responsible for their combinations with montmorillonite materials. Considering the work of these men and others as well as the appearance of the clay and juice after clarification, it seems probable that most of the organic colloids present are adsorbed by the clay particles and that these particles then coagulate with each other and sink through the juice thus occluding and sweeping out other particles. When the clay is stirred with the juice, an appreciable interval occurs during which no reaction seems to be taking place. Then the suspension suddenly "breaks," and the particles settle rapidly. Clarification is sufficiently complete so that the boiling off may be done in a relatively short time, with comparatively little skimming.

A detailed investigation of the theoretical factors influencing the clay clarification is extremely difficult. The mechanism of coagulation of clays by a consideration of the surface condition of the particles and the adsorbed ions which are present has been dealt with by Meyer (11) among others. It has been found (4) that clays which are apparently identical physically but differ in the nature of their clay mineral content will act very differently. Furthermore, even though the clays used are identical, a second difficulty is introduced by the fact that fresh sorgo

juice must be used if results are to be identical. Fresh juice may be obtained only during the rather short sorgo season. The juice decomposes very rapidly on standing even though toluene, chloroform, and low temperature storage are used to preserve it, the colloids coagulate and settle out to some extent, and this makes the juice very easy to clarify. Small-scale work is difficult to evaluate with regard to yield due to errors introduced in attempting to boil down and skim small batches of juice.

Practical tests were therefore developed by which different clays could be tested against each other, the various factors observed, and then large-scale runs made to determine the economic factors involved. Two apparently identical clays which gave considerably different results with regard to clarification were examined exhaustively to determine the causes of this difference in behavior.

## EXPERIMENTAL WORK

ACIDITY. The pH of fresh juice was found to be approximately 5. The acidities of the fresh juice, the clay clarified juice, and the final sirup were found to be practically identical.

PRECIPITATING AGENTS. It was found that 1 per cent sodium chloride, 1 per cent aluminum sulfate, 1 per cent sodium silicate and 2 per cent freshly precipitated silicic acid, added to separate samples of juice were not suitable colloid precipitants. When 0.6 gram of $Al_2(SO_4)_3$ was added to 100 cc. of juice and neutralized with the equivalent amount of $Na_2CO_3$ the resulting juice was water-white, but the precipitate took a long time to settle out.

Cider is often clarified by use of a gelatin-tannin mixture (7). It was found that by varying the ratio of gelatin to tannin according to acidity, the juice could be satisfactorily clarified, but the resulting sirups had bad color and taste. "Pectinol" (9) was found to be useless for the clarification of the juice.

## COMPARISON OF CLAYS

Preliminary work established the fact that "8 per cent" of clay (24 grams of dry pulverized clay per 300 cc. of juice) gave optimum results with a clay ("Hartford Clay") now being used successfully for commercial sirup production. The clay could be reused twice satisfactorily. However, under working conditions in warm weather, the clay may be used only twice in order to avoid spoilage of the juice. The following procedure was then adopted to determine empirically the usefulness of clays in general for juice clarification.

Twenty-four grams of pulverized clay were added to 300 cc. of fresh juice in a 500 cc. stoppered flask and immediately shaken to wet the clay. The flask was then shaken for 20 minutes and the contents, clay and all, were poured into a graduated cylinder. The rate and amount of settling and the volume of recoverable juice were noted and the clear

TABLE I

COMPARISON OF VARIOUS CLARIFYING AGENTS ON SORGO JUICE

| Sample No. | Type of Clarifying Agent Used | Rate of Settling | Color of Juice | Sirup Produced | Rating |
|---|---|---|---|---|---|
| 1 | Clay (Hartford, Iowa) | Very fast | Clear grey | Excellent | Excellent |
| 2 | Sandy loess (Redfield, Iowa) | Very fast | Very green | Discarded | Bad |
| 3 | Sandy Clay (Lehigh, Iowa) | Very fast | Very green | Discarded | Bad |
| 4 | Kaolin (Columbia, S. C.) | Slow | White | Excellent | Good |
| 5 | Bauxite Waste from Aluminum Co., (St. Louis, Mo.) | Did not settle | Red | Discarded | Bad |
| 6 | Mortar mix (Sheffield, Iowa) | Fast | Grey | Very good | Very good |
| 7 | Mortar mix (Adel, Iowa) | Fast | Grey | Very good | Very good |
| 8 | Brick Clay (Auburn, Iowa) | Fast | Green | Discarded | Bad |
| 9 | Crown Ball Clay (England) | Moderate | Clear grey | Very good | Good |
| 10 | Ball Clay (Kentucky) | Slow | Greenish grey | Poor | Poor |
| 11 | Vollendar Clay | Slow | Clear grey | Very good | Good |
| 12 | Kaolinite (Florida) | Slow | Clear grey | Excellent | Good |
| 13 | Limey Shale (Mason City, Iowa) | Slow | Clear grey | Excellent | Good |
| 14 | Shale (Des Moines, Iowa) | Fast | Clear grey | Very good | Very good |
| 15 | Bentonite ("Volclay") | Very slow | Clear grey | Good | Bad |
| 16 | Clay (Lynnville, Iowa) | Very fast | Clear grey | Excellent | Excellent |
| 17 | "No. 1263"* | Fast | Green | Poor | Poor |
| 18 | "No. 1316"* | Fast | Clear grey | Very good | Very good |

* These two clays are described in considerable detail in the text.

liquid siphoned off. The juice was then boiled down in a long narrow pan heated only at one end.

"Skimmings" were removed from the cold end by means of a fine wire screen. The juice was stirred near the end of the process to prevent scorching and was removed from the heat when the boiling point of the sirup was 111°C. The sirup was strained through the skimming screen and the turbidity, color, and taste observed. Although no quantitative results were possible, a satisfactory comparison could be made of the clays.

The results, using some Iowa clays and a few other available materials, are given in Table I.

From Table I it is evident that a number of clays are available for sirup clarification. Such clays can be obtained and their clarifying power determined by means of a practical test using fresh juice. The clay, of course, should give no objectionable taste to the juice. This may be ascertained by shaking the clay with water, allowing the clay to settle, and tasting.

## CHARACTERIZATION OF CLAYS

A very complete investigation was made of the clay minerals of two clays numbered "1263" and "1316" in Table I. Clay 1263 was from Marshall County, Iowa, and clay 1316 was from Cass and Pottawattamie counties, Iowa. Both were loess materials and very similar in appearance and physical properties. However, as shown in Table I, practical tests indicated that their reactions with sorgo juice were very different, and it was felt that a close examination of the clay minerals present might show why one could be used successfully for juice clarification and the other could not. The two clays were road subgrade materials which, although they could not be distinguished by the usual standard highway laboratory tests, showed considerable difference in behavior when used for road foundations, the sample 1263 being unsatisfactory (4).

The loess samples were put into suspension and the material less than one micron in diameter separated by sedimentation methods. The coarser material left was nearly all smaller than 270 mesh size. The fine material of 0.005 mm. or less amounted to 25 to 28 per cent of the total, and the material of 0.001 mm. in diameter (1 micron) amounted to approximately 15 per cent in each case. This fine material was then fractionated with a supercentrifuge, according to the methods of Bray, Grim, and Kerr (2), into three fractions: the coarse colloid, particles

TABLE II

RESUMÉ OF MECHANICAL ANALYSES BY SEDIMENTATION AND CENTRIFUGE

| | SAMPLE NO. 1263 | | SAMPLE NO. 1316 | |
|---|---|---|---|---|
| | Original Wt. 1,500 gms. | Percentage | Original Wt. 1,500 gms. | Percentage |
| Residue | 1,071.81 | 71.45 | 1,176.35 | 78.43 |
| Coarse colloid | 105.08 | 7.00 | 113.40 | 7.56 |
| Fine colloid | 37.67 | 2.51 | 46.36 | 3.09 |
| Superfine | 104.20 | 6.94 | 91.09 | 6.07 |
| Total colloids | 246.95 | 16.45 | 250.85 | 16.72 |
| Total colloids and residue | 1,318.76 | 87.90 | 1,427.20 | 95.15 |
| Lost in leaching and handling | 181.24 | 12.08 | 72.80 | 4.85 |

approximately 1 to 0.1 micron in diameter; the fine colloid, 0.1 to 0.06 micron; and the superfine colloid, all those less than 0.06 micron in diameter. These fractions were then studied microscopically, chemically, and by X-ray analysis.

A fractionation or mechanical analysis of material of this nature is essential mainly for three reasons:

1. To obtain an approximation of the size grade distribution of particles.

2. To separate the active or colloidal fraction from the relatively inert or non-colloidal fraction.

3. To facilitate microscopic study, X-ray analysis, chemical analysis,

TABLE III

X-RAY DATA*—COLLOID FRACTIONS

| | Coarse | | Fine | | Superfine | | d Values | |
|---|---|---|---|---|---|---|---|---|
| | 1263 | 1316 | 1263 | 1316 | 1263 | 1316 | A | |
| 1 | | M | | ? | ? | S | 13.64 | Montmorillonite |
| 2 | M | W | M | W | M | W | 9.85 | Illite |
| 3 | M | W | W | W | ? | | 7.25 | Kaolinite, Illite |
| 4 | WW | WW | W | W | W | W | 4.94 | $\beta$ Quartz |
| 5 | S | S | S | S | S | S | 4.49 | Clay** |
| 6 | S | S | WW | WW | | | 4.27 | Quartz |
| 7 | M | | W | W | | | 3.55 | Kaolinite, Quartz |
| 8 | SS | SS | S | S | | | 3.32 | Illite |
| 9 | M | M | | | | | 3.00 | Calcite |
| 10 | S | S | S | S | S | S | 2.59 | Clay** |
| 11 | M | S | | | | | 2.45 | Quartz |
| 12 | M | M | | | | | 2.28 | Quartz |
| 13 | W | W | | | | | 2.24 | Quartz |
| 14 | S | S | | | | | 2.12 | Quartz |
| 15 | M | M | WW | WW | | | 1.98 | Illite, Quartz |
| 16 | S | S | | | | | 1.81 | Quartz |
| 17 | W | W | W | W | W | W | 1.66 | Clay**, Quartz |
| 18 | S | S | | | | | 1.53 | Quartz |
| 19 | S | S | S | S | S | S | 1.49 | Clay** |
| 20 | S | S | | | | | 1.37 | Quartz |

* Observed intensities:
S.S. = Very strong
S. = Strong
M. = Medium
W. = Weak
W.W. = Very weak
** Not diagnostic of any particular clay mineral.

and base-exchange determinations by attempting to secure separates of relatively pure minerals.

The results of mechanical analyses by sedimentation and centrifuge are shown in Table II. The constituent clay minerals of the colloid fraction are best shown by the X-ray data in Table III which are in close agreement with the information gained through microscopic and chemical analysis; that is, they show that the principal difference between the two clays is the greater proportion of the clay mineral, montmorillonite, in sample 1316 than in sample 1263. The total base-exchange capacities of each of the colloidal fractions of the two samples are given in Table IV. The great difference in the base-exchange capacity between the colloidal and the noncolloidal fractions of both samples is striking. It is especially interesting to note the difference in the values of the superfine

TABLE IV

TOTAL BASE-EXCHANGE CAPACITY EXPRESSED AS MILLIEQUIVALENTS PER 100 GRAMS OF COLLOID

| | Residue | Coarse Colloid | Fine Colloid | Superfine Colloid |
|---|---|---|---|---|
| Sample 1263 ..... | 8.70 | 48.30 | 69.22 | 79.05 |
| Sample 1316 ..... | 13.20 | 45.31 | 65.98 | 95.24 |

colloid fractions of the two samples, sample 1316 having a noticeably higher value than 1263, due to the greater amount of montmorillonite present.

Inasmuch as the only significant difference between the two materials studied was the amount of montmorillonite present, and as montmorillonite is the significant clay mineral responsible for the adsorptive properties of a clay, it was concluded that the difference in the characteristic behavior of the two materials is due to this clay mineral content. This conclusion is in accord with the known facts of other high-grade commercial clarifying agents that are on the market. These agents, such as fuller's earth and bentonite clays, all contain the mineral montmorillonite which is believed to be responsible for the clarifying action.

However, for practical purposes, particle size also enters into the action. It was found that while the use of commercial bentonite, made up almost entirely of montmorillonite, resulted in a clear juice, the time required for gravity separation was so long as to be impractical. Apparently enough material of a particle size larger than that of the typical clay mineral must be present for fairly rapid settling. These larger particles probably act as nuclei around which the adsorptive clay mineral, montmorillonite, may cling. The importance of distribution of particle size as well as the distribution of the clay mineral species has been recognized in other fields, for example in paper coating clays (13),

and their influence in clarifying sorgo juice adds another interesting application.

## DESCRIPTION OF CLAY CLARIFICATION OF SORGO JUICE

Following is a description of the apparatus and process used, on a farm scale, in determining the loss of sirup by this method as compared with that lost when using the older process. The process is given in considerable detail so that the information may be of value to anyone desiring to use this procedure:

Sorgo cane juice is run from the mill through a screen into one of two wooden settling troughs 3½ feet wide, 7¾ feet long and 1 foot deep. (Deeper and narrower troughs would be advantageous since this would facilitate decanting the juice from the clay.) About 115 gallons of juice are run into the tank, and then the flow is diverted to the second tank. About 6-9 gallons (8 per cent of dry clay based on total weight of juice used) of thoroughly wet clay is then added to the juice and the muddy mixture is stirred for 10 to 20 minutes until it suddenly "breaks" and the coagulated clay mixture sinks to the bottom of the tank. The juice is allowed to settle for 20-30 minutes and is then decanted through a cloth bag by means of a swing pipe. The clay is reused on a second batch of juice. After the second use, a little water may be added to dilute the clay mixture and the liquid obtained added to the next tank-full. The clay is then discarded. The strained and clarified juice is now a light grey color and is run by gravity into the boiling pans. The pans used were of galvanized iron, 8½ feet long, 3 feet wide, and 13 inches deep. An unheated "dead end" 2¼ feet long and 10 inches deep was at one end of the tank. (The pans would be easier to use if they were 15 inches deep and if a longer dead end were provided.) The juice was evaporated by heat from steam coils made from 1-inch galvanized pipes, being boiled down to sirup in about 1 to 1.5 hours. A small amount of coagulated material ("skimmings") will collect at the cool end and should be skimmed off occasionally. When about two-thirds of the juice has boiled away, rapid boiling will produce a large amount of foam. This foam is easily dissipated by the addition of a small spoonful of lard. (Lard will *not* break the foam produced by juice which has not been clarified by the clay treatment.) When the boiling point has risen to 232°F. (111°C.), the sirup is done and may be run through a screen (about 40 mesh) into a cooling tank. Any clay sediment that is not strained out of the juice will ordinarily collect in the dead end of the evaporating pans.

The sirup obtained by this method is a light yellow liquid, clearer and lighter in color than any commercial product examined.

Sirup was also made by the old skimming process using juice from the same batch of cane and using the same equipment. Preliminary runs had shown that the loss was approximately 5 to 7 per cent greater by

the clay clarification method than by the older process but that this was more than compensated for by the improved quality of the sirup and the saving of time and labor.

The following is typical of the results obtained. The juice and sirup were carefully weighed and results were based on pounds of sugar, to compensate for unavoidable differences in specific gravities of the solutions. Samples were taken after each operation and immediately boiled to avoid fermentation. Allowance was made for any loss in weight dur-

TABLE V

COMPARISON OF SORGO SIRUP YIELD BY CLAY CLARIFICATION METHOD AND BY OLD SKIMMING METHOD

| SAMPLES TAKEN | OLD PROCESS | | CLAY CLARIFICATION PROCESS | | | |
|---|---|---|---|---|---|---|
| | | | RUN I | | RUN II | |
| | Weight in Pounds | Sugar Content Pounds | Weight in Pounds | Sugar Content Pounds | Weight in Pounds | Sugar Content Pounds |
| Raw juice ..... | 668.6* | 105.2 | 967.3† | 152.0 | 892.1‡ | 139.2 |
| Clay used (dry basis) ..... | ..... | ..... | 72.5 | ..... | 72.5** | ..... |
| Juice and clay left after clarification .. | ..... | ..... | 327.8 | 34.2 | 324.4 | 43.2 |
| Juice recovered from sludge for next run | ..... | ..... | 327.8 | 34.2 | 56.4 | 9.3 |
| Clay sludge discarded | ..... | ..... | none | none | 268.0 | 33.8 |
| Skimmings lost ..... | 20.5 | 6.2 | 3.0 | 1.1 | 4.0 | 1.8 |
| Sirup yield ..... | 142.8 | 100.4§ | 167.1 | 117.5‖ | 179.8 | 126.4¶ |
| Net loss ..... | ..... | 6.2 | ..... | 35.3 | ..... | 1.4 |
| Percentage loss ..... | 5.9 | | 23.2†† | | 1.0 | |
| Average loss per run | 5.9 | | 12.1 | | | |
| Average boiling time per gallon of juice in minutes ..... | 1.3 | | 0.76 | | | |

* 74.7 gallons of juice.
† 108.5 " " "
‡ 99.5 " " "
§ 12.3 " sirup (Sp. gr. 1.39)
‖ 14.3 " "
¶ 15.5 " "
** The clay of run I was re-used.
†† Includes the loss put over into the second run.

ing boiling. The samples were then analysed for total sugar by the Shaffer and Hartmann method (14), and results were calculated to the same total sugar basis. Results of a typical run are given in Table V.

The percentage loss was figured on the actual amount of sugar lost rather than upon the sirup recovered because this could be measured more accurately. From the data presented in Table V, it is evident that about 6 to 7 per cent more of the total yield of sirup will be lost in the

clay method as compared to the other method. This loss is a variable in that it is dependent upon the juice left in the sludge at the end of the run. As this sludge is re-used in succeeding runs, the loss becomes greatly reduced. Furthermore, more than 80 per cent of the feed value of the skimmings will be lost. To counterbalance this, however, is the great saving in time and labor and the increased value of the product. No allowance has been made in Table V for recovery of juice by washing the clay. It was found that after washing the clay, it was possible to recover an amount of liquid exactly equal to the volume of wash water used. Since the amount of sorgo juice lost in the clay is approximately equal to 24 gallons, washing with 24 gallons of water would give a 50 per cent mixture of juice and water, thus reducing the loss appreciably. The increased cost of evaporating the excess water would, of course, counterbalance this to some extent. Probably washing with 12 gallons of water, which should recover about eight gallons of juice, would be profitable and would reduce the average loss per run almost 4 per cent to about 8 per cent, or only 2 per cent more than the older method.

One of the greatest advantages of the clay process over the older process is the great saving of time. When batches are run as indicated in Table V, the time of boiling is cut almost in half. Also, larger batches may be run because foaming is greatly reduced when boiling is rapid. Since the boiling-down period is the "bottleneck" of the process as carried out on the farm, this saving is a very important factor in favor of the clay process. The shortness of the sorgo season necessarily limits investment to very simple, rather low-cost equipment, since such apparatus must stand idle a great part of the year. The acreage of sorgo to be planted must be rather carefully considered in order to be able to process all of the crop before freezing weather arrives. In such cases the clay-clarification method shows its worth, since the capacity of a plant is approximately doubled because of the rapidity with which the clarified juice may be boiled down. The tedious labor of continuous skimming is avoided, and, although the sirup must be tended closely, the workman is free to do a considerable amount of incidental work around the sirup house. Finally, the product is so much improved in appearance and in flavor that the increased selling price which it should command would easily make up for the 6 per cent loss.

## CONCLUSIONS AND SUMMARY

It has been found that Iowa clays may be used to clarify sorgo juice for the production of sorgo sirup on the farm. Such sirup is of equal or better quality, with regard to taste, clarity, and color, as compared to that produced by other methods. A description of the process and a practical method of testing clays for their clarifying power is given. An exhaustive examination of two clays of very similar appearance, but which had a considerably different effect upon the clarification of sorgo

juice, showed that the principal difference was that the clarifying agent contained the clay mineral, montmorillonite. Examination of a number of clays indicated that particle size was also an important factor in the practical use of the clay for clarification since the clay must settle fairly rapidly after clarification. Clays suitable for this use are fairly widely scattered in Iowa.

It was found that although the yield of sirup is about 6 per cent less by this method, the saving in time and labor and the improved quality of the product amply compensate for this loss.

## LITERATURE CITED

1. Bartling, William
1930. Production of Sorghum Sirup. Facts about Sugar, 35, No. 10: 38-42.

2. Bray, R. H., R. E. Grim, and P. F. Kerr
1935. Technique for the Investigation of Argillaceous Sediments. Bulletin Geological Society of America, 46:1909-1926.

3. Bryan, A. H., and S. F. Sherwood
1924. Sorgo-Sirup Manufacture. United States Department of Agriculture, Farmers' Bulletin No.1389,10-38.

4. Cuthbert, F. L.
1940. Petrography of Two Iowa Loess Materials. The American Mineralogist, 25:519-527.

5. Demolon, A., and G. Barbier
1929. Conditions de formation et Constitution du Complexe argilo-humique des Sols. Compt. Rend. Acad. Sci., 188:654-656.

6. Ensminger, L. E., and J. E. Giesking
1939. The Adsorption of Proteins by Montmorillonite Clays, Soil Science, 48:467-474.

7. Fabian, F. W., and R. E. Marshall
1935. How to Make, Clarify, and Preserve Cider. Circular Bulletin No. 98 (Revised), Michigan Agr. Exp. Sta.

8. Gaessler, W. G., J. D. Reid, and F. L. Cuthbert
1940. Short Cuts in Sorghum Making. Iowa Farm Science Reporter, Vol. I, No. 3: 12-13.

9. Lantz, H. L.
1934. Modern Methods of Cider Making. Transactions Iowa State Horticultural Society, 69:28-32.

10. Mattson, S.
1932. The Laws of Soil Colloidal Behavior: VII. Proteins and Proteinated Complexes. Soil Science, 23:41-72.

11. Meyer, W. W.
1937. Nature of China Clays. Paper Trade Journal, 105, No. 11, 155-156.

12. Meyers, H. E.
1937. Physiochemical Relations between Organic and Inorganic Soil Colloids as Related to Aggregate Formation. Soil Science, 48:467-474.

13. Rowland, B. W.
1940. The Colloidal Nature of Clay with reference to Papermaking. Paper Trade Journal. 111, No. 16, 207-212.

14. SHAFFER, P. A., AND A. F. HARTMAN
The Iodometric determination of Copper and its use in Sugar Analysis. I and II, J. Biol. Chem., 45:349-390.

15. VENTRE, E. K., AND S. BYALL
1937. Distribution and Variation with Maturity of Dissolved Solids, Sucrose and Titratable Acidity in the Sorgo Stalk. Journal of Agricultural Research, 55:553-562.

16. WALTON, JR., C. F., E. K. VENTRE, AND S. BYALL
1938. Farm Production of Sorgo Sirup. U.S.D.A., Farmers' Bulletin No. 1791, 1-39.

17. WILEY, H. W.
1893. Record of Experiments with Sorghum in 1892. U.S.D.A., Division of Chemistry, Bulletin No. 37, 84-85.

18. WILLAMAN, J. J., AND S. S. EASTES
Factors Affecting Color in Sorghum Sirup. J. Industrial and Engineering Chemistry, 21:1138-1145.

# THE FRUCTOSAN CONTENT OF SOME GRASSES ADAPTED TO IOWA[1]

## A preliminary survey

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The presence of fructose polysaccharides in members of the Gramineae has been reported at various times and names such as graminin, triticin, etc., have been given to these inulin-like carbohydrates (2). Archbold (1) has recently reviewed the literature in this field. Most attention has perhaps been given to the fructosans of cereals, such as barley and wheat, in the plants of which polysaccharides of this nature appear as a transitory reserve in amounts which rarely exceed 2 to 3 per cent. Certain grasses under the climatic conditions of England have been shown to contain, at an intermediate stage of growth, much larger amounts (4, 5). In rye grass (Western wolths) a maximum of over 30 per cent of the dry weight of the aerial part of the plant was found at the heading stage, equivalent to about 400 lb. fructosan per acre. As much as 43 per cent was present in the first internode above the surface at this time. As the plant matured, however, the fructosan content was found to fall rapidly, probably by conversion to structural constituents. Finally, at maturity, the fructosan content of rye grass was only 3.2 per cent. Young second growth rye grass did not contain as high a content of this polysaccharide as first growth material of approximately equal nitrogen content and stage of growth. A diminishing fructosan content was found in orchard grass or cocksfoot (*Dactylis glomerata*) though the peak attained was only 11 per cent, this is the youngest sample (6). The mature grass in this case, however, contained as much as 8 per cent fructosan. Nitrogen fertilization had the result of reducing fructosan accumulation on a percentage basis. Observations of a similar nature were made in the U.S.S.R. by Morosov (3) on *Bromus inermis, Lolium perenne,* and *Festuca pratensis.*

The survey reported herein was made in the summer of 1939 with the intention of ascertaining whether any grasses adapted to Iowa conditions contain at some stage of growth sufficient fructosan to justify the study of its commercial extraction to supplement the inulin process as a source of fructose. With this aim in view it was only justifiable to examine grass samples taken at a stage when the dry weight yields per acre were appreciable. Inasmuch as previous work had pointed to the time of heading as

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the period at which fructosan might be expected to reach its highest level, most of the grasses were first sampled as they neared this stage. Second cuts were taken in some cases.

## METHODS

*Samples.* The grass samples were collected at the Agronomy Farm, Ames, and put in a hot air drier within two or three hours to inactivate enzymes. Grinding to about 50 mesh was carried out in a Christie and Norris mill.

The following grass species were analyzed: Brome grass (*Bromus inermis*), Kentucky bluegrass (*Poa pratensis*), Reed canary grass (*Phalaris arundinacea*), Orchard grass (*Dactylis glomerata*), Domestic (western) ryegrass (*Lolium multiflorum*), and Sudan grass (*Sorghum vulgare* var. *sudanense.*

*Fructosan determination.* The procedure followed involves extraction, hydrolysis, and determination of reducing power after removal of aldoses by oxidation to the corresponding aldonic acids. This may only be considered a satisfactory method in the absence of sucrose, the fructose component of which would be determined as fructosan.

A 3-gram sample was extracted with 100 ml. water in a boiling water bath for 30 minutes. The residue after filtration was thoroughly washed with hot water and the filtrate and washings combined in a 250-ml. volumetric flask, to which was added 3 ml. saturated neutral lead acetate solution before making up to volume. Fifty ml. clear supernatant was pipetted into a 100-ml. flask, and 1 ml. 10 per cent disodium phosphate added to precipitate the excess lead. Full precipitation occurred on neutralization with sodium hydroxide, a drop of phenolphthalein being added to determine the end point. After filtration 50 ml. were hydrolyzed under reflux by boiling with 1 g. dry oxalic acid. Partial neutralization was effected by addition of precipitated calcium carbonate. Ten ml. of the cold filtrate were placed in a 25-ml. volumetric flask, and neutralization was completed with sodium hydroxide if necessary. To this was added 2 ml. 0.1 N iodine (put into solution with 34 g. potassium iodide per liter) and 1 ml. 1 per cent sodium hydroxide. The flask was shaken, stoppered, and put in a refrigerator, not over 10°, for 2 hours. After acidification by addition of 1 ml. 0.3 N sulfuric acid, the excess iodine was removed by titration with sodium sulfite. The concentration of the sulfite is immaterial, except that not over 7-8 ml. should be needed. A burette with a fine jet was used and one drop dilute starch solution added to obtain the end point. After completion of this titration the excess sulfuric acid present was neutralized by 0.1 N. sodium hydroxide, and the volume made up to 25 ml. Five ml. of this solution were heated with 5 ml. of the Shaffer-Somogyi copper reagent in the usual way (7). The sugar content was read off on a curve previously obtained. It is to be noted that the reducing value of fructose is influenced by the presence of iodide, and unless this be taken into account, appreciable error may be introduced. The final sugar titrations were run

in triplicate, one being used to establish quickly the approximate endpoint and the second and third to determine it accurately.

The dilution system indicated above (1:250) is satisfactory for low fructosan concentrations, but may be varied in one or more places if larger amounts are present. Dilution after removal of the excess iodine remaining from the hypoiodite oxidation may be to 50 ml. or 100 ml. if necessary,

TABLE I

REDUCING POWER OF FRUCTOSE IN THE PRESENCE OF IODIDE, USING SHAFFER-SOMOGYI REAGENT 50 CONTAINING 150 ML. 0.1N IODATE PER LITER

| FRUCTOSE MGM. IN 5 ML. | TITRATION VALUE IN ML. 0.005 N. THIOSULFATE | | | |
|---|---|---|---|---|
| | Without Added Iodide | 0.4% Iodide (25 ml. Volume) | 0.2% Iodide (50 ml. Volume) | 0.1% Iodide (100 ml. Volume) |
| 1.194 | 10.55 | 9.95 | .... | .... |
| 1.160 | 10.23 | 9.60 | 9.83 | 9.95 |
| 0.910 | 7.98 | 7.35 | 7.58 | 7.72 |
| 0.575 | 5.02 | 4.38 | 4.65 | 4.83 |
| 0.287 | 2.45 | 1.95 | 2.10 | 2.30 |
| 0.132 | 1.18 | 0.93 | .... | .... |

but in such an event a different curve must be used for ascertaining the sugar present because of the reduced iodide content. Data for constructing the necessary curves are given in Table I.

## RESULTS AND CONCLUSIONS

The fructosan contents of the various grass samples are given in Table I. Fructosan was found in all species, but the amounts even in young growth were relatively small, in most cases being of the order of 2-3 per cent. The highest content found was 7.6 per cent in first growth sudan grass at the time of blooming. For this reason second growth sudan was sampled at intervals of three to four days, but at no stage was this figure exceeded, the peak of 6.6 per cent being reached before heading.

On the basis of this survey the conclusion must be reached that the fructosan content of grass species adapted to Iowa conditions is not sufficiently high to justify the hope that they might serve as a supplementary source for the preparation of fructose.

The results do, however, raise certain questions concerning the physiology of grasses, and their carbohydrate transformations of which little is known. The fructosans appear to be ubiquitous reserves in grasses, but perhaps only of a transitory nature. Their accumulation and disappearance may be related to growth conditions since there was, for example, a greater change in percentage content in sudan grass samples taken but two or three days apart than could be accounted for by changes in total dry weight. Of the two species previously studied in England, namely ryegrass and orchard grass, the former is not well suited to Iowa conditions, and the latter is not commonly grown. Accordingly, only three

TABLE II

FRUCTOSANS IN IOWA GRASSES, 1939, EXPRESSED ON THE OVEN DRY BASIS

| Grass Species | Date Cut | Plant Height Inches | Notation | Fructosan Percentage* |
|---|---|---|---|---|
| SUDAN (*Sorghum vulgare* var. *sudanense*) | May 29 | 6 | Young spring growth. | 3.67 |
| | June 9 | 16 | Leafy growth. | 5.18 |
| | June 20 | 27 | Leafy growth, no heads. | 2.33 |
| | June 26 | 33 | Heads emerging. | 5.41 |
| | July 3 | 36 | In bloom. | 7.60 |
| | June 20 | 21 | Second growth since May 29 harvest. | 2.58 |
| | July 3 | 8 | Second growth since June 26 harvest. | 4.00 |
| | July 11 | 16 | Second growth since June 26 harvest; leafy. | 5.34 |
| | July 14 | 18 | Second growth since June 26 harvest; leafy. | 6.62 |
| | July 19 | 27 | Second growth since June 26 harvest; leafy. | 4.08 |
| | July 21 | 36 | Second growth since June 26, no heads | 4.14 |
| | July 24 | 38 | Second growth since June 26; heads beginning to emerge. | 3.29 |
| | July 26 | 40 | Second growth since June 26; many plants in head. | 2.50 |
| | July 29 | 43 | Second growth since June 26; plants starting to bloom. | 2.77 |
| | July 31 | 44 | Second growth since June 26; plants in bloom. | 4.02 |
| | Aug. 2 | 51 | Second growth since June 26; plants in full bloom | 4.07 |
| | Aug. 5 | 51 | Second growth since June 26; late bloom | 2.79 |
| SMOOTH BROME-GRASS (*Bromus inermis*) | May 2 | 11 | Fresh young growth. | 2.89 |
| | May 29 | 16 | Plants starting to bloom. | 1.69 |
| | May 29 | 14 | Second growth since May 2 harvest. | 1.91 |
| KENTUCKY BLUEGRASS (*Poa pratensis*) | May 2 | 8 | Young leafy growth. | 3.57 |
| | May 29 | 4 | Second growth since May 2 harvest. | 3.02 |
| | June 9 | 4 | Second growth since May 15 harvest. | 3.82 |
| | June 20 | 8 | Second growth since May 15 harvest. | 2.12 |
| | July 11 | 11 | Second growth since June 1 harvest. | 3.76 |
| ORCHARD GRASS (*Dactylis glomerata*) | May 29 | 27 | Plants heading. | 2.54 |
| REED CANARY (*Phalaris arundinacea*) | May 2 | 9 | Fresh young growth. | 2.55 |
| | May 29 | 11 | Leafy growth; no heads. | 3.01 |
| | June 9 | 14 | Many heads emerging. | 3.11 |
| | May 29 | 8 | Second growth since May 2 harvest. | 2.38 |
| DOMESTIC RYEGRASS (*Lolium multi-florum*) | June 9 | 5 | Young growth; leafy. | 3.60 |
| | June 20 | 8 | Heads starting to emerge. | 2.93 |

* Expressed as total fructose after hydrolysis.

samples of them in all were taken. The fructosan content of these was far lower than occurs at approximately the same stage under English conditions. Similarly, the samples of brome grass analyzed by Morosov (3) contained considerably more fructosan than the three samples reported herein.

## SUMMARY

A survey was made of the fructosan content of some of the more important grass species adapted to Iowa conditions in order to ascertain whether any of these might serve as a source for the preparation of fructose. In almost all samples the content was of the order of 2 to 4 per cent, which would not be sufficiently high for this purpose. The highest amount found, 7.6 per cent, was in sudan grass at the blooming stage.

## REFERENCES

1. Archbold, H. K.
1940. Fructosans in the monocotyledons: A review. New Phyt. 39:185-219.

2. Cugnac, A. de
1931. Les glucides des graminées. Importance de fructoholosides. Bul. soc. chim. biol., Paris, 13:125.

3. Morosov, A. S.
1939. Storing of carbohydrate by forage grasses. Compt. rend. (Doklady) Acad. Sci. U.R.S.S. 24:407-409.

4. Norman, A. G.
1936. The composition of forage crops. Rye grass (Western wolths). Biochem. J. 30:1354-1362.

5. Norman, A. G., and H. L. Richardson
1937. The composition of forage crops. II. Rye grass (Western wolths). Changes in herbage and soil during growth. Biochem. J. 31:1556-1566.

6. Norman, A. G.
1939. The composition of forage crops. III. Cocksfoot. Changes in the herbage during growth, with and without additions of nitrogenous fertilizer. Biochem. J. 33:1201-1208.

7. Shaffer, P. A., and M. Somogyi
1933. Copper-iodometric reagents for sugar determination. J. Biol. Chem. 100:695-713.

# STUDIES ON THE MYXOBACTERIA[1]

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## 1. Distribution in Central Iowa With Description of a New Species

As a consequence of the development of satisfactory techniques for the isolation of myxobacteria, the Krzemieniewskis (1926) found that many species previously thought to be more or less obligate coprophiles could be cultivated from the soil. In much of their work they relied solely on soil samples and were able not only to re-isolate many species that had been previously found only on dung, but to discover several new species. In undertaking the present work a modification of the Krzemieniewski isolation technique was employed.

### METHODS OF ISOLATION

Samples of soil, and of dung of various kinds, from several localities, were collected in small large-mouthed bottles fitted with screw caps. The samples were taken from the upper four or five centimeters of the soil since experience had shown that very few myxobacteria were to be found below that depth. Dung that had lain on the ground for some time was found to harbor larger numbers of myxobacteria than fresher material. The soil, or dung, after being collected, was broken up into small particles. Sieving of the soil was often resorted to in order to eliminate stones, grass, and other undesirable materials.

Petri dishes were prepared by placing two or three pieces of filter paper in the bottoms, to aid in retaining the moisture, and enough of the sample added to approximately half fill the plate. Twenty-five to forty pieces of fresh rabbit dung were then placed on top of the soil, or dung, and water added up to 75 to 90 per cent saturation. Various incubation temperatures were tried, but the most satisfactory was found to be room temperature, 20° - 25° C. When held there the development of fruiting bodies on the dung was more normal, though possibly a little slower, than at a somewhat higher temperature.

During the first three to four days, molds developed, in some cases in such large numbers as to make the plates of no further use. Usually the molds reached maximum growth within five days, after which fruiting bodies of some species of myxobacteria began to develop. Plates not completely overgrown by molds were examined daily under a low-power binocular microscope, and when myxobacterial fruiting bodies

[1] Journal Paper No. J-862 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 571.

were found they were transferred to plates of dung decoction agar that had previously been prepared.

This medium was made by adding to 100 grams of fresh rabbit dung one liter of distilled water. The mixture was heated to boiling, then allowed to infuse for 24 hours, filtered through several layers of cheese cloth, the filtrate made up to volume, and 1.5 per cent Bacto agar added. Sterilization was effected by autoclaving at 15 pounds pressure for 30 minutes. When cooled to about 60° C. plates were poured.

The primary transfers, of which 127 were made from the various soils and dungs, were, of course, not pure cultures. Often molds were carried along on the needle, and these frequently outgrew the myxobacteria. Associated bacteria of various types were also present and usually developed first. These, too, occasionally grew rapidly enough to completely inhibit the growth of the more slowly growing slime bacteria. However, fruiting bodies were sometimes able to develop, and were first seen on the tops of colonies of the associated types. Attempts to transfer parts of these fruiting bodies to new media usually resulted in transferring both the myxobacteria and the associate. The appearance of some of these primary transfers after seven to ten days incubation is shown in Figures 1, 2, and 4. The fruiting bodies are seen to be developing on the surface of colonies of associated types. By allowing a longer period of incubation pure cultures could be obtained from the thin, nearly transparent vegetative slime colony that developed beyond the margins of the colonies of associated bacteria. Two weeks incubation were often required for the development of these vegetative colonies, and the fruiting bodies that eventually formed on them were usually smaller and less perfect than those produced directly on the host colony. This fact may be noted in Figure 1. These observations led to the development of a somewhat improved method for isolation.

After molds had been eliminated, fruiting bodies were transferred to a medium made by growing large numbers of true bacteria (*Escherichia coli, Bacillus subtilis, Serratia marcescens,* etc.) in flasks of nutrient broth. After a good growth had been obtained the suspensions were shaken well, in order to distribute the cells evenly throughout the suspension, and 1.5 per cent agar added. The flasks were then autoclaved at 15 pounds pressure for 30 minutes. Transfers were then made to plates of this medium. The growth of the myxobacteria was greatly enhanced, and the colonies of vegetative cells developed much more rapidly than did those of associated types. A later modification of this method consisted of growing large numbers of true bacteria on agar, scraping the growth from the agar and washing free of any adhering nutrient materials. An angle-head centrifuge was used for the purpose. After three washings in distilled water the cells were concentrated, the supernatant liquid poured off, and the paste dried *in vacuo.* The dried cells were then suspended in 1.5 per cent plain agar (no other nutrient

materials were added), and the medium sterilized as usual. This medium produced vigorous growth of the myxobacteria, while the associated species failed, in nearly every case, to develop at all. This procedure shortened the isolation period to a few days, rather than two or three weeks.

When pure cultures had finally been obtained they were transferred to dung plates. These were prepared by placing two or three pieces of rabbit dung in each petri dish and sterilizing for 1 hour at 15 pounds pressure in the autoclave. A 1.5 per cent solution of Bacto agar in distilled water was added to each plate to a depth equal to about one-half the diameter of the pellets of dung. The dung was then moved to the center of the plates with a sterile needle or forceps before the agar solidified. Care was taken not to cover the dung, but to keep the top side of each piece free from agar. Much better growth could be had directly on the dung than on agar-incrusted dung. Transfers were made to the dung, the agar acting to hold it in place and to preserve the moisture content. Growth of all species was found to be good on this medium, and the fruiting bodies produced were large and appeared more nearly normal than on most other media that had been tried. This medium has been used successfully for carrying stock cultures over periods of many months. Transfers are necessary about once every six to eight weeks, depending upon humidity and temperature.

## SPECIES ISOLATED

Undoubtedly conditions vary sufficiently from one country to another to affect, to a high degree, the flora of the soils. It has been the experience of the present author that the majority of types to be found in the soils in the environs of Ames, Iowa, belong to the genus *Myxococcus* and that other types, while present, are not abundant. Private communications with two other persons interested in the group tend to indicate that this is also true in other parts of the United States. Many of the species used for this work were originally found on dung.

Of the 127 primary transfers made, only a small percentage were finally isolated in pure condition. This was chiefly due to the fact that the associated organisms (both bacteria and molds) were able to outgrow the myxobacteria after transfer from the natural substrate. It is of interest to note the variety of types that were observed, with some indication of the natural substrate. A condensed list is given here, with brief descriptions of the species that were isolated in pure culture. These are indicated by the culture numbers following the names.

## SPECIES CULTURED

*Myxococcus fulvus* (Cohn *emend.* Schroeter) Jahn 1, 44, 76, 106. Produces bright pink fruiting bodies, spherical to subspherical, often constricted at the base, and supported on a mound, or foot, of slime; the surface smooth with no outer wall or limiting membrane; up to 350 μ in

diameter. Spores spherical, average 1.3 μ diameter.

From wet sand, pasture soil, cow, sheep, and goat dung.

*Myxococcus fulvus* var. *albus* Jahn 108. Similar to *M. fulvus* but color much lighter, often giving rise to very pale pink or nearly white fruiting bodies.

*Myxococcus virescens* Thaxter 57, 61, 91, 100. Fruiting bodies somewhat smaller and less regularly spherical than those of *M. fulvus*. Color yellow to greenish yellow. Spores 2.0 μ diameter.

From wet sand, pasture soil, goat dung.

*Myxococcus xanthus*[2] Beebe 115, 127. Similar to *M. virescens* in size and shape of fruiting bodies and spores. Color bright orange.

From pasture soil and cow dung.

*Polyangium fuscum* (Schroeter) Thaxter 77. Fruiting bodies composed of masses of oval to spherical cysts, each surrounded by a tough, reddish-brown wall; up to 90 x 125 μ. Mass of cysts held together by a colorless, transparent slime envelope. Resting cells, or spores, rod-shaped; about 0.8 x 3.0 μ.

From sand, sandy loam, pasture soil, cow, sheep, and rabbit dung.

*Podangium erectum* (Schroeter) Jahn 82, 104. Reddish-brown cysts, oval or club-shaped, rising from a sort of hypothallus. Cysts single, each with a thick outer wall or membrane; 40 - 50 μ in diameter, up to 100 μ in height. Often 100 or more on one hypothallus. Spores shortened rods, 0.7 x 3.0 μ.

From cow dung and soil from woods and pasture.

**Chondrococcus Blasticus, sp. n. 90, 111.**

This organism was at first considered a large variety of *M. fulvus* due to the marked similarity of the primary fruiting body of this species, both in color and formation, and those of *M. fulvus*.

ETYMOLOGY: Greek adj. = budding, additional growth.

## DIAGNOSIS

FRUITING BODY. Primary: Spherical to subspherical, usually sessile but occasionally with a short stalk or foot, pale to bright pink; 300 to 600 μ in diameter. No outer wall or limiting membrane evident. Develops on sterilized rabbit dung in from 3 to 5 days at room temperature. Secondary: Arising as a budlike growth from the primary fruiting body. Develops into irregularly shaped, finger, coral, or budlike protuberances. Seldom branched, occasionally stalked but usually sessile on primary fruiting body. Deep pink to salmon pink in color. Quite variable in size and shape: 50 - 150 x 175 - 425 μ. No outer wall or limiting membrane evident.

[2]Diagnosis of *M. xanthus* included in paper submitted to Journal of Bacteriology; in press at the time of this writing.

SPORES. Spherical, thick-walled, highly refractile; 1.2-1.4 μ in diameter. Held together in the fruiting body by the mass of slime.

VEGETATIVE COLONY. Thin, colorless, transparent at margin; surface broken by many small ridges, or veins. Center smoother, slightly thicker, often showing pale pink pigmentation.

VEGETATIVE CELLS. Long, slender, flexible rods, straight or curved to bent, ends rounded to tapered, gram negative. Often show one or two deep-staining bodies within, at or near center, while ends of cell stain lightly. 0.5 - 0.6 x 3.0 - 5.0 μ. Usually found in groups of from 2 to 12, lying parallel, the group moving as a unit. Motile by a crawling or creeping motion; no flagella.

HABITAT. Observed once on goat dung, once in soil, Ames, Iowa.

## SPECIES IDENTIFIED BUT NOT CULTURED

*Chondromyces crocatus* Berkeley and Curtis from cow dung.

*Chondrococcus coralloides* (Thaxter) Jahn from pasture soil and cow dung.

*Chondrococcus* sp. (yellow) from sheep dung.

*Myxococcus* sp. (deep red-orange) from sheep dung.

*Myxococcus fulvus* and *M. virescens* were found to be by far the most numerous species, the fruiting bodies appearing in large numbers on plates of nearly every sample brought into the laboratory. *Polyangium fuscum* was noted on a wide variety of soils and dungs, but always in small numbers, as was the case with *M. xanthus* and *Podangium erectum*. *Chondromyces crocatus* appeared in moderately large numbers on only one sample of cow dung. Attempts to purify this species failed completely, as was the case with *Chondrococcus coralloides* and undetermined species of *Chondrococcus* and *Myxococcus*. *Myxococcus fulvus* var. *albus* was found only once in sandy soil.

Several authors have reported various species of myxobacteria from such materials as bark of trees, dead leaves, straw, etc. These materials were all sampled during the course of the work but no slime bacteria were isolated from any of them.

## SUMMARY

An improved method for the isolation of myxobacteria from soils and dungs is described.

A new species, *Chondrococcus blasticus*, is diagnosed.

The distribution of species of the *Myxobacteriales* in Iowa soils in the vicinity of Ames is discussed and shown to be fairly general as regards the families *Myxococcaceae* and *Polyangiaceae*. By far the most common species were those belonging to the genus *Myxococcus*, particularly *M. fulvus* and *M. virescens*. *M. xanthus* also appeared on a wide variety of soils and dungs, but in smaller numbers. This was also the case with *Polyangium fuscum* and *Podangium erectum*. No species of

the families *Sorangiaceae* or *Archangiaceae* were observed, though it is not suggested that they are entirely absent from the soils of this region.

## LITERATURE CITATIONS

BERKELEY, M. J.
Intro. Crypt. Bot. 313, London; 1857.

JAHN, E.
Beiträge zur botanischen Protistologie. I. Die Polyangiden. Geb. Borntraeger, Leipzig; 1924.

KRZEMIENIEWSKI, H. AND S.
Miksobakterje Polski (Die Myxobakterien von Polen). Acta Soc. Bot. Pol., 4(1):1-54; 1926.

SCHROETER, J.
Schizomycetes in Cohn's Kryptogamenflora von Schlesien, 3(1):170; 1889.

THAXTER, ROLAND
On the Myxobacteriaceae, a new order of Schizomycetes. Bot. Gaz., 17:389-406; 1892.

———
A new order of Schizomycetes. Bot. Gaz., 18:29-30; 1893.

———
Further observations on the Myxobacteriaceae. Bot. Gaz., 23:395-411; 1897.

———
Notes on the Myxobacteriaceae. Bot. Gaz., 37:405-416; 1904.

## PLATE I

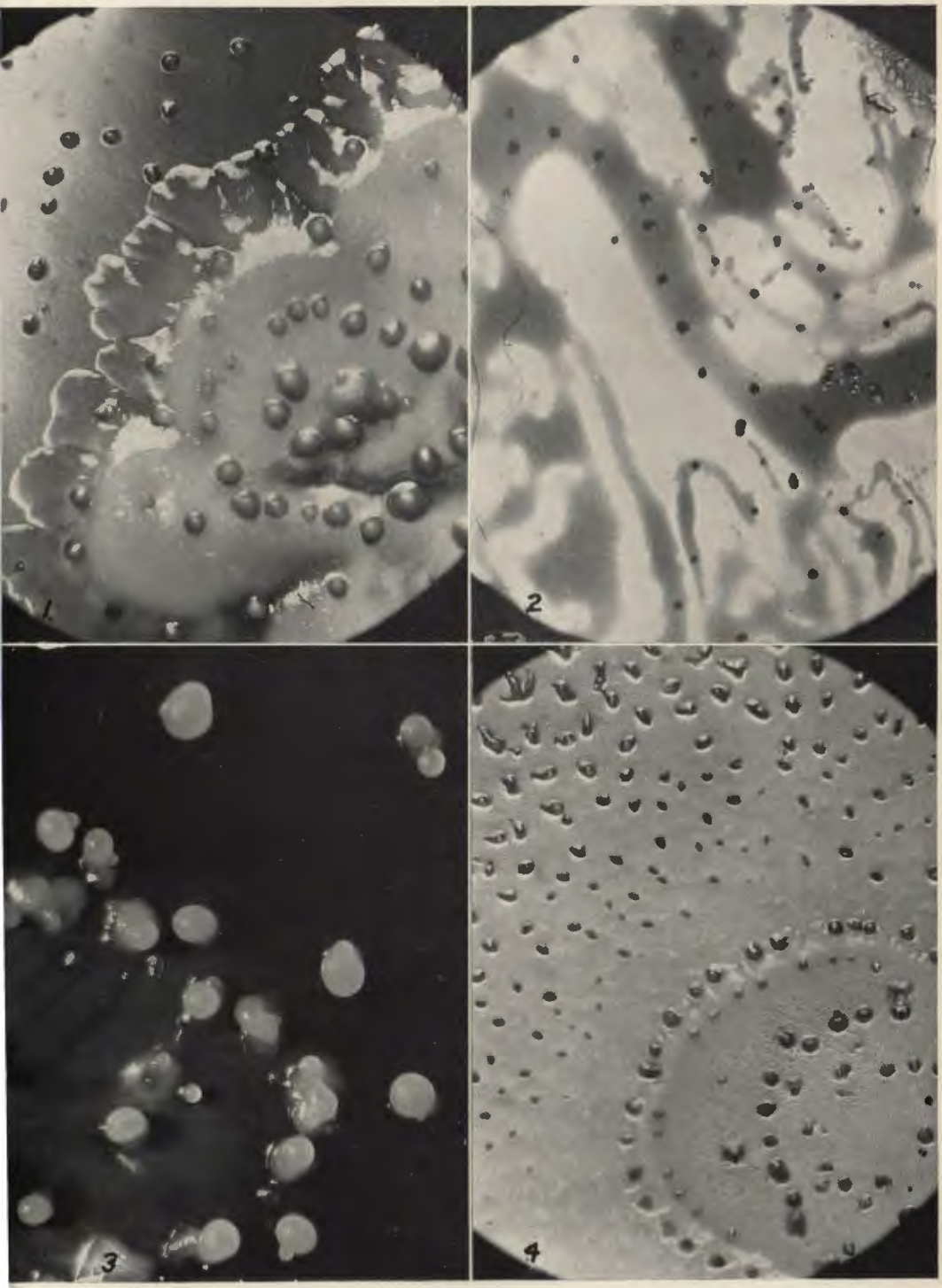
1. Fruiting bodies of *Myxococcus fulvus* produced on a host colony. The vegetative myxobacterial colony may be seen to extend beyond the margin of the host colony, and to have formed smaller fruiting bodies directly on the agar. 20 ×.

2. *M. fulvus* fruiting bodies on a different type of host colony. Note the tendency of the fruiting bodies to form on the eubacterial host colony rather than on the agar. 10 ×.

3. Pure culture of *M. fulvus* growing on rabbit dung plate. The fruiting bodies shown are on the surface of the agar around the imbedded piece of dung. 20 ×.

4. *Myxococcus virescens* growing on and around a colony of true bacteria. 10 ×.

PLATE I



## PLATE II

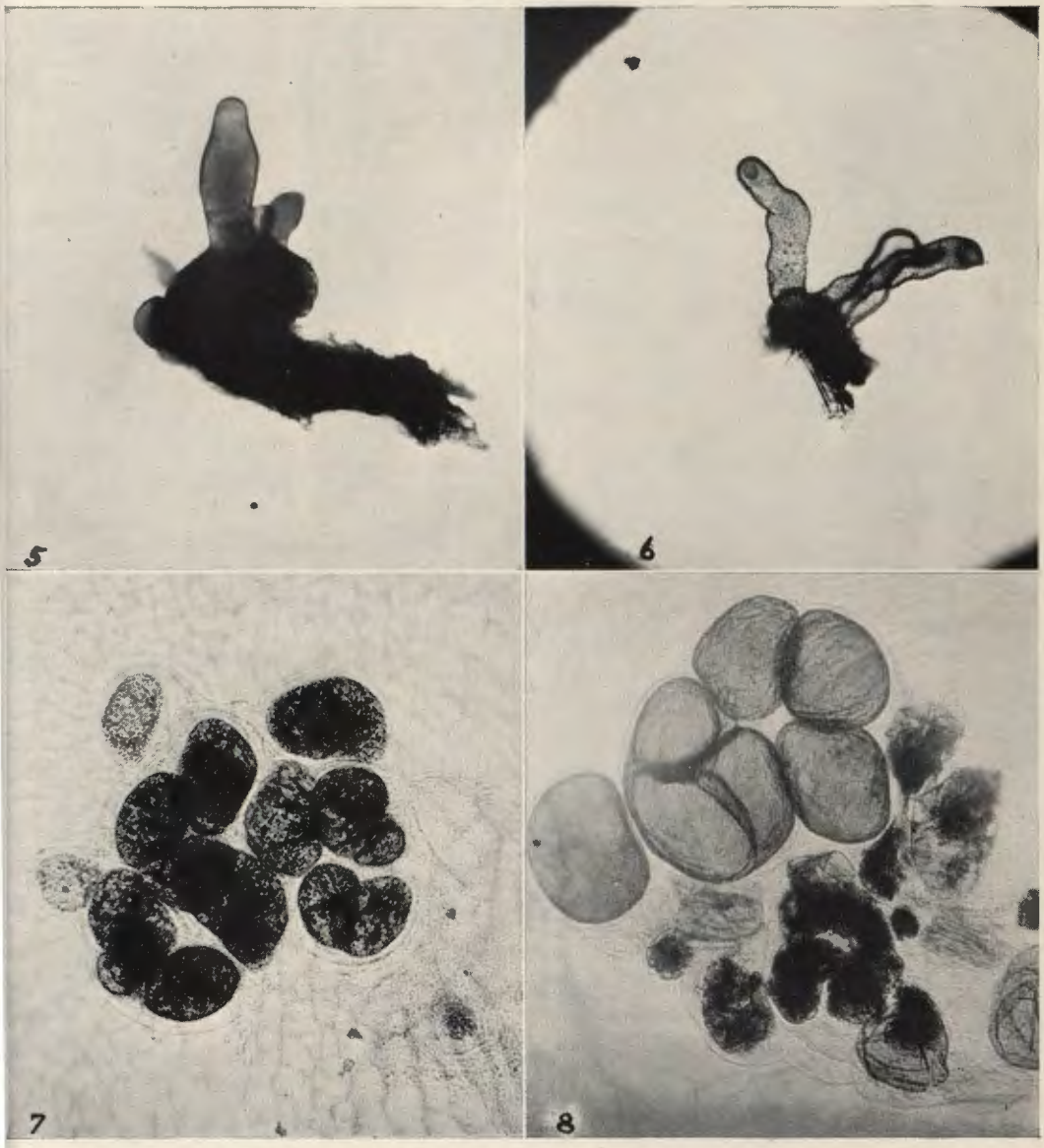
5. Immature fruiting body of *Chondrococcus blasticus* sp. n. The secondary fruiting bodies are shown developing from the large primary. On sterilized rabbit dung. 50 ×.

6. Mature fruiting body of *C. blasticus*. The large primary fruiting body has been completely utilized in the formation of the secondaries. 50 ×.

7. Several stages in the formation of cysts of *Polyangium fuscum*. The fruiting body first forms as a mass of colorless slime containing the long, flexible, rod-shaped bacteria. The cells then begin to group themselves at various points to form cysts, which become differentiated within the slimy mass. An early stage is shown near the lower right corner of the illustration; the cyst wall has not yet formed. Near the opposite corner are cysts in a more advanced stage, the cyst wall forming, but as yet unpigmented. Cysts near the center are nearly mature and show large pigmented areas in the walls. 125 ×.

8. Mature cysts of *P. fuscum*. These are much larger than those shown in Fig. 7, and the pigment is evenly distributed throughout the walls. The slime envelope which holds the mass of cysts together is shown in both photographs. About 150 ×.

PLATE II

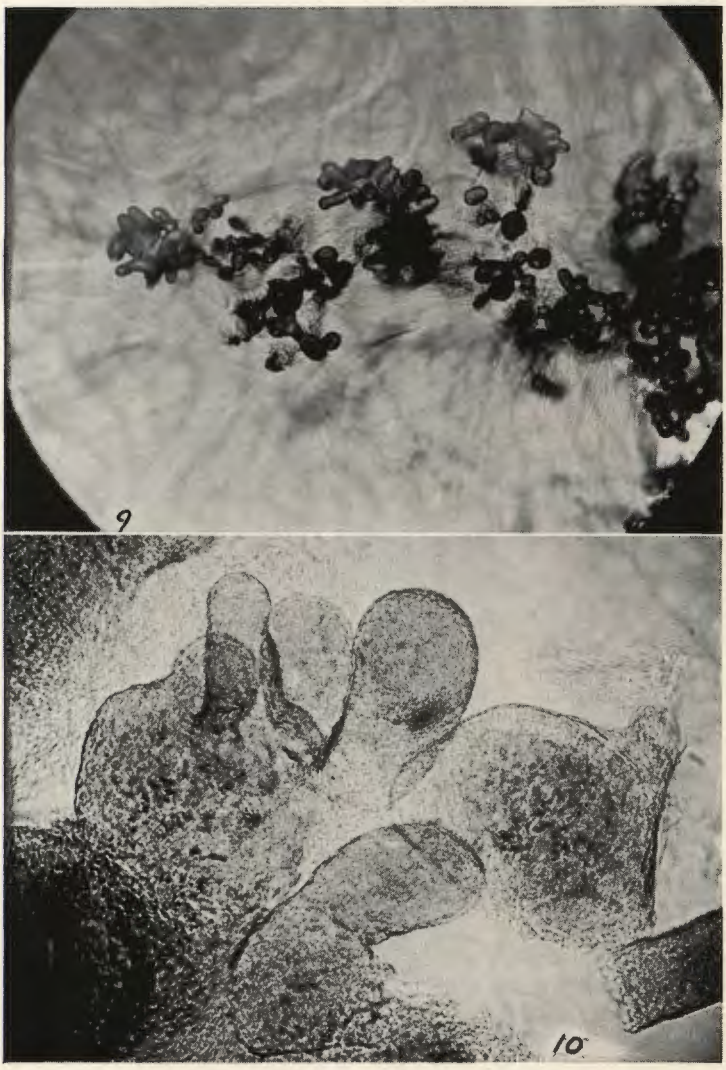


## PLATE III

9. Clumps of fruiting bodies of *Podangium erectum* growing on agar. Oval or club-shaped cysts may be noted. 50 ×.

10. Cysts of *P. erectum* cleared in xylol and mounted in immersion of oil. The moderately thick cyst walls may be seen, as well as the shortened, rod-shaped cells within. 430 ×.

PLATE III



## STUDIES ON THE MYXOBACTERIA

### 2. The Role of Myxobacteria as Bacterial Parasites

Few of the investigators who have concerned themselves with the myxobacteria have studied the nutritional requirements of the members of this group. The earlier workers observed that most species developed well on dung of one kind or another. Quehl (1906) recorded slow growth of some species which he cultivated on malt extract gelatine, the gelatine becoming liquefied. Good growth occurred on potato nutrient agar, but sterilized potato was not a satisfactory substrate. Kofler (1913) described good growth on a sucrose-peptone agar containing small amounts of magnesium sulphate and potassium phosphate. He regarded peptone as necessary for normal development.

Pinoy (1913) made first mention of a possible symbiotic or parasitic relationship of certain myxobacteria with species of true bacteria. He noted that *Chondromyces crocatus* failed to develop on any medium unless some species of *Micrococcus* was also present. He concluded the presence of the latter was necessary for the growth of the former.

In a report on the utilization of various carbon compounds by certain species of the *Myxobacteriales,* Beebe (1940) showed the use of both starch and cellulose, the former being broken down by a diffusible extracellular enzyme. He also noted a dependence of the myxobacteria on other bacterial forms: killed suspensions of true bacteria were found to supply all of the nutrient requirements of the slime bacteria. This was suggested as being parasitism rather than symbiosis, and was borne out by the findings of Snieszko, McAllister and Hitchner (1941) who pointed out a lytic action of certain species of myxobacteria on living colonies of gram-negative true bacteria.

The purpose of the present work was to determine, if possible, something of the nature of the nutritional demands, with special emphasis on the relationship with true bacteria, of several species of myxobacteria from soils and dungs of Iowa.

#### ISOLATION PROCEDURES

The methods used in the isolation of the various species have been previously outlined. In brief they were as follows:

Soil and dung samples, collected in screw-cap jars, were pulverized and sieved, and distributed in petri dishes to a depth of about one-quarter inch. Twenty-five to forty pieces of rabbit dung were placed on the sample and water added to about 75 to 90 per cent saturation. The plates were incubated at room temperature (22° - 25° C.) for ten to fourteen days. Molds developed rapidly the first three or four days of incubation, but after their maximum period of growth had passed,

fruiting bodies of the myxobacteria began to form on the dung. The plates were examined daily under a low-power binocular microscope. When fruiting bodies were located they were transferred to plates of dung decoction agar. If the fruiting body germinated, and was not overgrown with associated molds and bacteria, an additional transfer to a bacterial cell suspension agar usually completed the isolation procedure. Stock cultures were held on plates of sterilized rabbit dung partially imbedded in plain 1.5 per cent agar.

## ASSOCIATED BACTERIA

Forty-six isolations were made of the organisms found associated with the myxobacteria in order to determine whether any particular type played an important part in the growth and development of the slime bacteria. Of the total number only five were spore-forming rods; two were cocci. The remainder were gram-negative, non-spore-forming rods varying in shape from nearly spherical cells less than a micron long up to rods nearly 4 μ long. In only a very few cases did the pigmentation vary decidedly from white or light yellow; one culture produced pale orange pigment, a few developed a bright yellow color after several days, and two were pale pink or pinkish orange. Except in the case of the cocci, which belonged to the genus *Staphylococcus*, and in the case of one spore-former, the cells were all single, never forming chains or any other definite type of group. On the whole they represent common soil forms; they have not as yet been further identified.

Several of these associated forms were used during the succeeding work. Each has been identified by a number indicating its source, such as Associate No. 100/3: the third associate cultured from primary isolation No. 100.

## EXPERIMENTAL PROCEDURES AND RESULTS

Early observations showed what appeared to be a close relationship between the myxobacteria and certain true bacteria. Growth of such organisms as *Chondromyces crocatus* in mixed culture, especially in the presence of various bacteria and molds, and the ability of this organism to develop only poorly, if at all, under other conditions, checked closely with the work of Pinoy (1913) on the same species. *Myxococcus virescens* also showed a marked tendency to degenerate when in pure culture, but to revive and produce more or less normal fruiting bodies when again transferred to unsterilized rabbit dung. *Polyangium fuscum* presented a problem in isolation in that it appeared to thrive in what seemed to be an extreme case of association: a very small, motile, gram-negative, non-spore-forming rod was noted to live in the slimy outer envelope surrounding the ripened cysts, as well as in the slime of the vegetative colony. Both organisms seemed to benefit by the association and it was only after many months that a separation was effected. The associate failed to develop on any other medium. *P. fuscum* was found to

grow well without it when inoculated on sterilized rabbit dung, or a bacterial suspension in agar. The development of large, perfectly formed fruiting bodies by most of the species on the tops of colonies of associated true bacteria, while those developing beyond the margins of the host colonies were smaller and less perfect, also indicated the importance of the relationship.

However, it was felt that some other factor might have an important role in the nutrition of the slime bacteria; the frequent reference of the earlier workers to growth on dung of all kinds might mean the utilization of fecal types of bacteria by the myxobacteria, the presence of some sort of growth factor in dung (aside from bacteria) particularly favorable for the growth of myxobacteria, or a combination of both.

After several preliminary trials a series of experiments was run in an effort to determine whether the dung itself was necessary to the growth and development of certain species, or whether the factor influencing the myxobacteria was to be found in the bacteria said to compose a large part of dung. Four media were used: (1) nutrient agar containing the usual 3 grams of beef extract, 5 grams of peptone, and 15 grams of agar per liter; (2) dung decoction agar, made as previously described; (3) bacterial suspension-nutrient agar which was prepared by inoculating 1 liter of nutrient broth with an actively growing broth culture of associate No. 91/3, a large, non-spore-forming rod. This was incubated at 37.5° C. for 48 hours, during which time it was shaken frequently for purposes of aeration. 1.5 per cent agar was then added and the suspension autoclaved for 30 minutes at 15 pounds pressure. This medium contained both bacterial cells and their metabolic products. (4). Bacterial suspension agar was prepared by growing the same organism, No. 91/3, on large slants (1 x 8 inch-tubes) of nutrient agar. After three days incubation at 37.5° C. the heavy growth of 20 slants was removed, suspended in distilled water used for washing the slants clean, and concentrated by centrifugation. The cells were re-washed and centrifuged twice in order to remove any adhering nutrient materials or metabolic products. The washed cells were suspended in 250 cc. of distilled water and plate counts made to compare the concentration of cells with that of the bacterial suspension-nutrient agar medium, plate counts having been previously made on the latter. The cell counts on the washed suspension averaged about twice those made on the nutrient broth suspension. Accordingly, the washed cell suspension was diluted to twice its volume, 500 cc., and 7.5 grams of agar added. Sterilization was in the autoclave at 15 pounds pressure for 30 minutes.

All of these media were used for plate cultures. Inoculations were made by transferring matured fruiting bodies from the stock cultures, rabbit dung plates, by means of a fairly short needle. In order to facilitate the transfer a low-power binocular microscope was used; this made it possible to pick up about the same amount of material on the needle

for each transfer, and eliminated, to a large degree, carrying over bits of dung or agar from the stock cultures. The fruiting bodies were implanted on the centers of the plates. Incubation for this first work was at 30° C. for periods of from six to ten days. As a rule 12 to 72 hours were required for the germination of the spores before any growth could be noted; this period depended chiefly on the species, rather than temperature or medium.

The following species were used in this experiment:

*Myxococcus fulvus* 44, 76.
*M. virescens* 57, 61.
*Polyangium fuscum* 77.
*Podangium erectum* 82, 104.
*Chondrococcus blasticus* 90, 111.

The results after seven days are shown in Tables I - IV. Growth was compared, by measuring the diameters of the colonies in millimeters, each day during the incubation period; formation of fruiting bodies is indicated by figures in boldface.

TABLE I

DIAMETERS OF COLONIES IN MILLIMETERS ON BACTERIAL SUSPENSION AGAR SEVEN DAYS—30.0° C.

| NAME AND NUMBER | AGE IN DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| *M. fulvus* 44 ..... | 12.5 | 20.5 | 28.0 | **35.0** | **40.0** | **46.0** |
| *M. virescens* 57 ..... | 6.4 | 16.0 | 25.0 | **30.0** | **38.0** | **47.0** |
| *M. virescens* 61 ..... | 6.4 | 13.5 | 20.0 | **25.0** | **28.0** | **33.0** |
| *M. fulvus* 76 ..... | 14.0 | 22.5 | 32.0 | **36.0** | **42.5** | **46.0** |
| *P. fuscum* 77 ..... | 8.5 | 8.5 | 10.0 | ..... | ..... | ..... |
| *P. erectum* 82 ..... | 3.5 | 5.0 | 10.0 | 17.0 | 23.0 | **30.0** |
| *C. blasticus* 90 ..... | 7.2 | 16.0 | 23.0 | 30.0 | 33.0 | **38.0** |
| *P. erectum* 104 ..... | 3.2 | 9.5 | 18.0 | 30.0 | 38.0 | 45.0 |
| *C. blasticus* 111 ..... | 12.0 | 18.0 | 25.0 | **30.0** | 34.0 | 40.0 |

TABLE II

DIAMETERS OF COLONIES IN MILLIMETERS ON NUTRIENT AGAR SEVEN DAYS—30.0° C.

| NAME AND NUMBER | AGE IN DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| *M. fulvus* 44 ..... | 6.5 | 10.0 | 15.0 | 17.5 | 20.0 | 22.5 |
| *M. virescens* 57 ..... | 5.6 | 12.0 | 18.0 | **23.0** | **27.0** | **33.0** |
| *M. virescens* 61 ..... | 6.4 | 10.0 | 15.0 | **18.0** | **21.0** | **25.0** |
| *M. fulvus* 76 ..... | 6.4 | 9.0 | 11.5 | 12.5 | 13.0 | 14.0 |
| *P. fuscum* 77 ..... | 9.5 | 10.0 | 10.0 | ..... | ..... | ..... |
| *P. erectum* 82 ..... | 1.5 | 1.6 | 5.0 | 11.0 | 16.5 | 24.0 |
| *C. blasticus* 90 ..... | 3.6 | 6.0 | 8.5 | 10.0 | 11.5 | 14.0 |
| *P. erectum* 104 ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| *C. blasticus* 111 ..... | 4.5 | 8.0 | 13.0 | 14.5 | 16.0 | 17.5 |

TABLE III

DIAMETERS OF COLONIES IN MILLIMETERS ON BACTERIAL SUSPENSION-NUTRIENT AGAR SEVEN DAYS—30.0° C.

| NAME AND NUMBER | AGE IN DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| *M. fulvus* 44 | 10.0 | 13.0 | 20.0 | ..... | ..... | ..... |
| *M. virescens* 57 | 9.0 | 14.0 | 21.0 | 26.0 | 28.5 | 33.0 |
| *M. virescens* 61 | 9.6 | 15.0 | 18.0 | 25.0 | 30.0 | 36.0 |
| *M. fulvus* 76 | 8.5 | 11.5 | 15.0 | 16.0 | 17.5 | 18.0 |
| *P. fuscum* 77 | ..... | ..... | ..... | ..... | ..... | ..... |
| *P. erectum* 82 | 3.2 | 3.5 | 3.5 | 12.0 | 19.0 | 25.0 |
| *C. blasticus* 90 | 6.0 | 13.0 | 20.0 | 24.0 | 26.0 | 28.0 |
| *P. erectum* 104 | ..... | ..... | ..... | ..... | ..... | ..... |
| *C. blasticus* 111 | 8.5 | 15.0 | 21.0 | 25.0 | 29.0 | 32.0 |

TABLE IV

DIAMETERS OF COLONIES IN MILLIMETERS ON DUNG DECOCTION AGAR SEVEN DAYS—30.0° C.

| NAME AND NUMBER | AGE IN DAYS | | | | | |
|---|---|---|---|---|---|---|
| | 2 | 3 | 4 | 5 | 6 | 7 |
| *M. fulvus* 44 | 1.6 | 2.0 | 3.0 | 6.0 | 7.0 | 8.0 |
| *M. virescens* 57 | 3.6 | 4.5 | 8.0 | 11.0 | 14.0 | 21.0 |
| *M. virescens* 61 | 5.6 | 8.0 | 13.0 | 17.0 | 20.0 | 27.0 |
| *M. fulvus* 76 | 5.5 | 8.0 | 11.0 | 15.0 | 18.0 | 23.0 |
| *P. fuscum* 77 | 6.0 | 6.2 | 7.0 | ..... | 21.0 | 31.0 |
| *P. erectum* 82 | 3.6 | 4.5 | 5.2 | 7.0 | 7.5 | 7.5 |
| *C. blasticus* 90 | ..... | ..... | ..... | ..... | ..... | ..... |
| *P. erectum* 104 | 3.5 | 3.7 | 7.0 | 15.0 | 20.0 | 34.0 |
| *C. blasticus* 111 | 2.4 | 2.4 | 2.4 | ..... | ..... | ..... |

In order to compare more easily the growth of any one organism on the four media, the figures for several of the cultures given above have been plotted as growth curves. These are given in Figures 11 to 16. With two strains of *Myxococcus fulvus* better growth was obtained on the suspension of killed cells alone. In culture No. 44 the second best growth was on the nutrient agar-bacterial suspension combination, followed by plain nutrient agar and lastly by dung decoction agar. With culture No. 76 the three last named media closely approximated each other, all being much less favorable than the bacterial suspension alone. *Myxococcus virescens* 57 and 61 presented less striking pictures, although in one case there is a significant difference between the colony size on the suspension of killed cells and that on the nutrient agar, and bacterial suspension-nutrient agar, both of which appeared to produce identical results. Dung decoction agar ranked third in the case of No. 61, fourth in the case of No. 57. *Polyangium fuscum* 77 failed to grow in three instances, developing only moderately well on dung decoction agar. *Podangium erectum* 82 and 104 showed particularly significant differences in rates of growth on bacterial suspension agar and dung

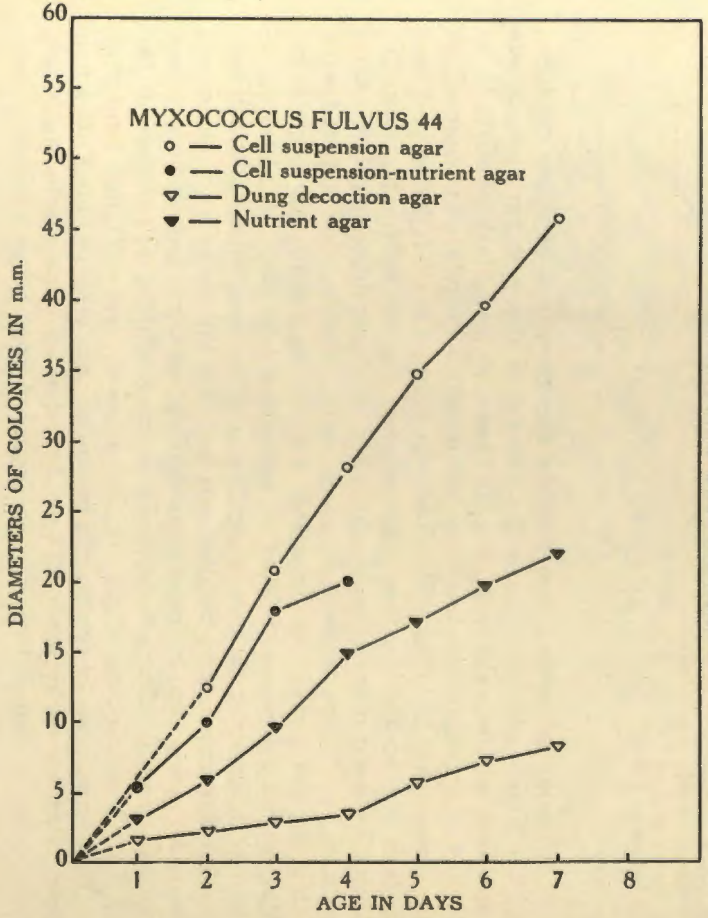

MYXOCOCCUS FULVUS 44
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 11

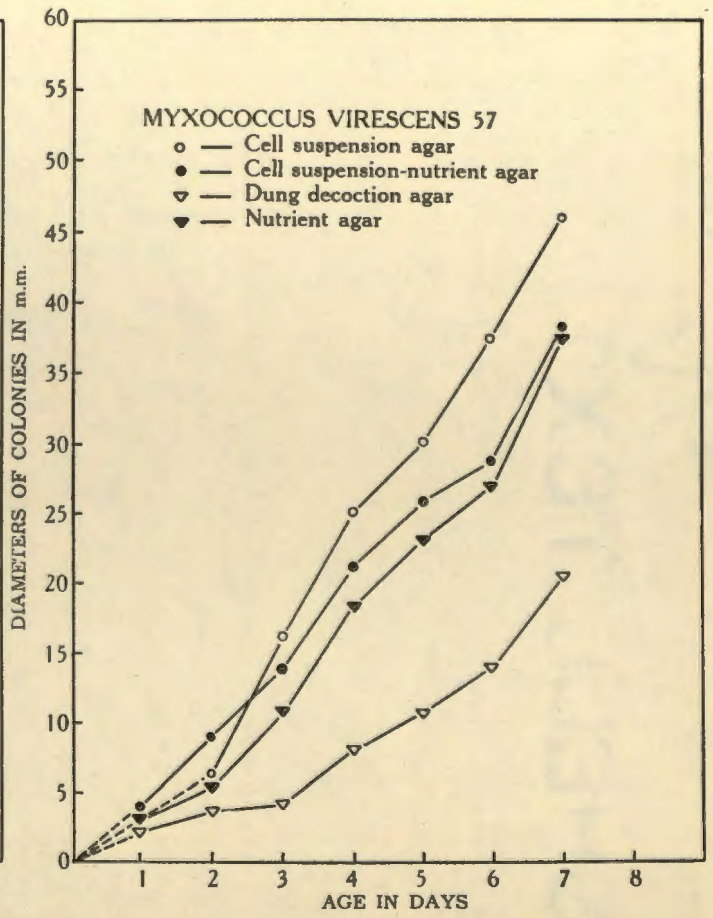

MYXOCOCCUS VIRESCENS 57
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 12

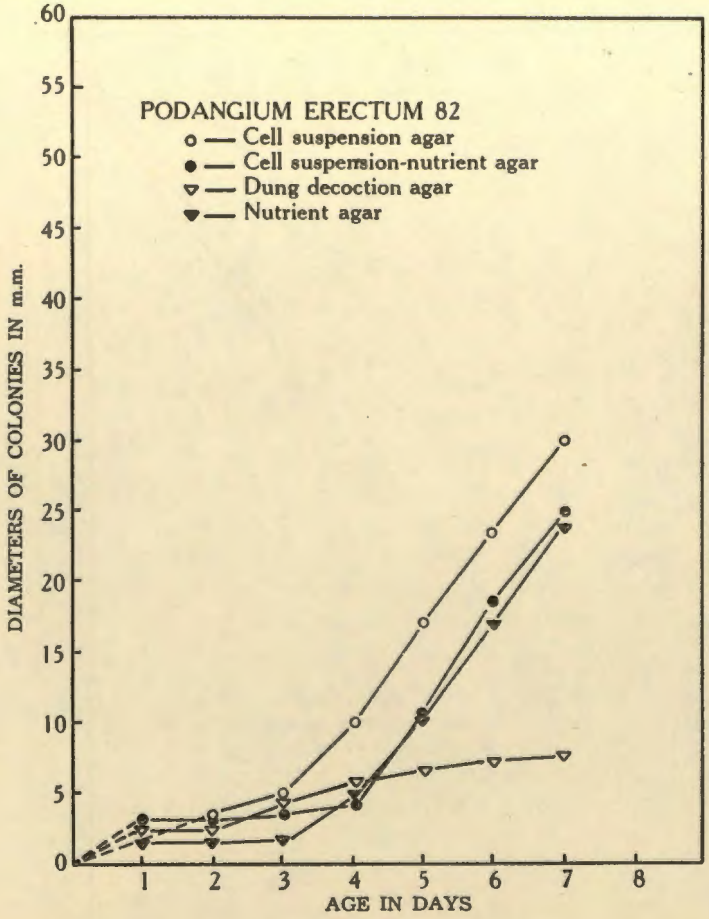

PODANGIUM ERECTUM 82
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 13

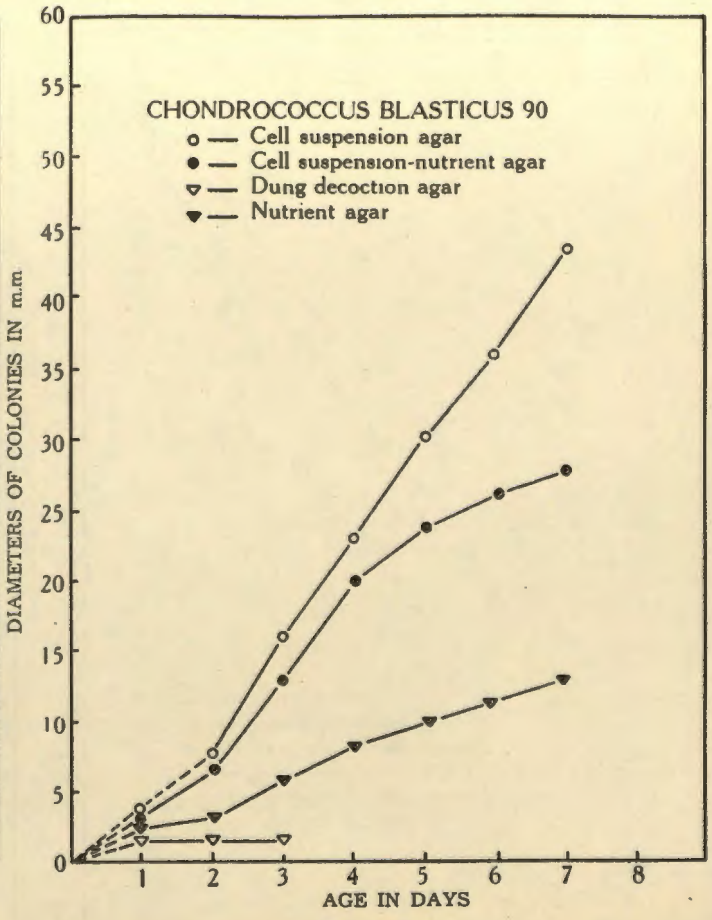

CHONDROCOCCUS BLASTICUS 90
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 14

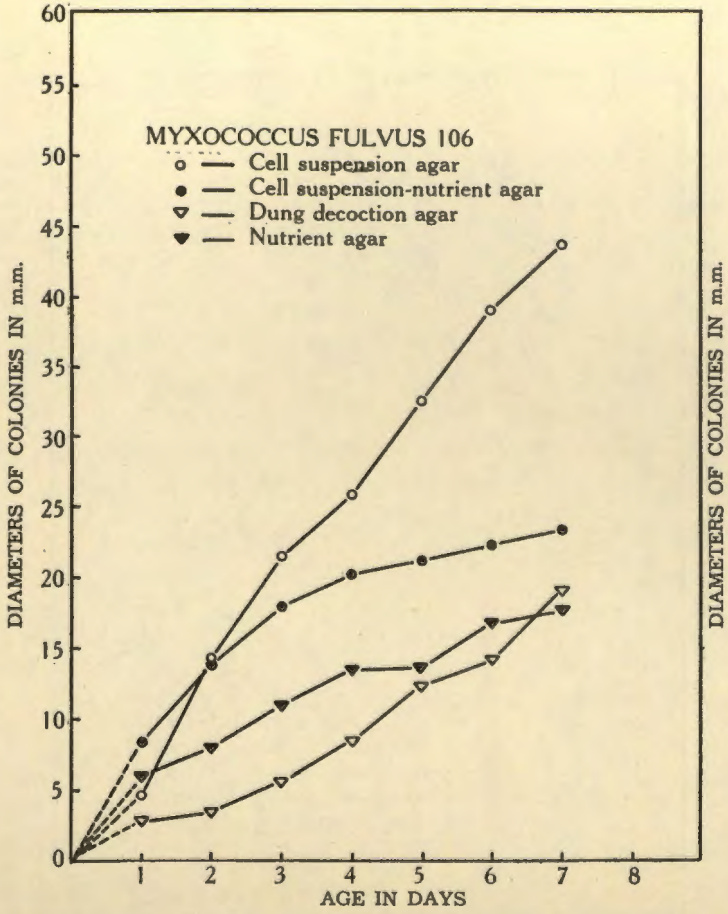

MYXOCOCCUS FULVUS 106
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 15

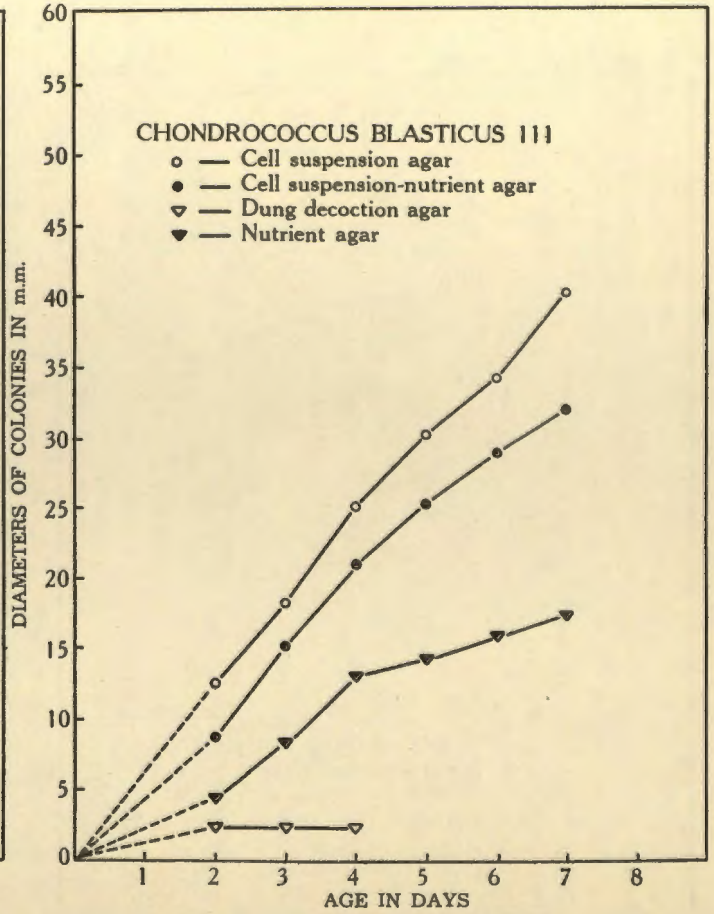

CHONDROCOCCUS BLASTICUS 111
Cell suspension agar
Cell suspension-nutrient agar
Dung decoction agar
Nutrient agar
DIAMETERS OF COLONIES IN m.m.
AGE IN DAYS
0
5
10
15
20
25
30
35
40
45
50
55
60
1
2
3
4
5
6
7
8

FIG. 16

decoction agar. Culture number 82 gave identical results on the two nutrient agars, while 104 failed to develop on either. *Chondrococcus blasticus* 90 and 111 both grew rapidly, in a nearly straight line curve, on the suspension of killed cells alone, with no apparent decrease in rates of growth during the entire incubation period. On the nutrient agar-cell suspension medium there is seen to be a definite falling off in rate after 4 days, while the same is true on nutrient agar. The entire latter curve is much lower than either of the preceding. This organism failed to grow on dung decoction agar.

The production of fruiting bodies may also be considered indicative of the value of a particular medium. Seven of the nine strains produced fruiting bodies on cell suspension agar at the end of the period of incubation, while one, *M. fulvus* 76, had fruited by the third day. Five produced fruiting bodies by the fifth day. At the end of the seven-day run only three cultures showed fruiting bodies on nutrient agar, and two on each nutrient-cell suspension and dung decoction agars.

A second series of experiments was run to check the first. This time associates number 82/1 and 100/3, a short, gram-negative rod and a *Staphylococcus* respectively, were used in the suspensions. It was thought that the presence of the metabolic products might have some inhibitory effects on the growth of the myxobacteria so that the cells to be suspended were grown on large slants, the growth removed as previously described, and re-suspended in distilled water to which was added 7.5 grams of plain agar in one case, 11.5 grams of prepared Difco Bacto Nutrient agar in the other, to each 500 cc. of suspension. The results, given in condensed form in Table V, after seven days incubation at 30° C., were essentially the same as in the previous trials.

TABLE V

DIAMETERS OF COLONIES IN MILLIMETERS ON FOUR CELL SUSPENSION MEDIA SEVEN DAYS—30.0° C.

| NAME AND NUMBER | ASSOCIATES | | | |
|---|---|---|---|---|
| | 82/1 | | 100/3 | |
| | PLAIN | NUTR. | PLAIN | NUTR. |
| *M. fulvus* 44 | 17 | ..... | 15 | ..... |
| *M. virescens* 57 | 27 | 19 | 19 | 25 |
| *M. virescens* 61 | 24 | 27 | 26 | 28 |
| *M. fulvus* 76 | 22 | 6 | 17 | 12 |
| *P. fuscum* 77 | 31 | 19 | 23 | ..... |
| *P. erectum* 82 | 42 | 28 | 37 | 24 |
| *C. blasticus* 90 | 25 | 10 | 22 | 10 |
| *P. erectum* 104 | 37 | ..... | 15 | 9 |
| *C. blasticus* 111 | 26 | 13 | 21 | 14 |

Except for *M. virescens* 57 and 61, best growth invariably occurred on the suspension of short rods (No. 82/1), and the next most favorable medium in every case was the suspension of *Staphylococcus* No. 100/3.

A comparison of the two nutrient agars will show little difference with the type of suspended cells. Fruiting body production was higher on the two plain cell suspensions, although there was less difference to be seen here than in the previous trials.

Within 24 hours after the inoculations on the suspensions of cells, a clarified area, or window, could, in most cases, be noted around the point of inoculation. As the colonies grew, this area was seen to increase in size. The cells in the suspensions appeared to be lysed by extracellular enzymes secreted by the myxobacteria. Microscopic examination of agar from within this area showed very few cells remaining in the medium, while the agar outside the growth area revealed the presence of numerous cells intact. In a few cases the lysed area extended several millimeters beyond the margin of the myxobacterial colony, but for the most part the diameters of the two coincided.

Quantitative determinations were then made in an effort to show the effect of varying the concentrations of the cell suspensions. Trial runs, using associate culture No. 100/3 again, indicated a direct relationship between the number of cells in the suspension and the rates of growth of the myxobacteria. In these first experiments the cells for the suspensions were grown on large slants and suspended in distilled water to give concentrations of 80,000,000; 40,000,000; and 20,000,000 cells per cubic centimeter. These were determined by plate counts. No other nutrient materials were added to the suspension in agar. Fruiting body production on the three media was, in general, about the same throughout. This was thought to be due to too little difference between the highest and lowest cell concentrations.

A series of more closely controlled experiments followed. It was felt that better control could be exercised with definite weights of cells rather than approximate numbers such as result from plate counts. Large numbers of cells were grown in flats—six-ounce Blake bottles to which had been added 20.0 cubic centimeters of nutrient agar; these were plugged with cotton, sterilized as usual, and allowed to cool in a horizontal position, giving a large surface of nutrient agar upon which to grow the cells. Inoculations were made by pipette from actively growing 24-hour broth cultures, and the flats incubated at 37.5° C. for two days. The growth was then scraped off, suspended in distilled water, centrifuged, and then re-washed and centrifuged two additional times. The resulting cell paste was dried *in vacuo* and stored in a desiccator over calcium chloride.

The following cultures were so treated and used for cell suspensions:

No. 100/4—Large spore-forming rods, colorless.
No. 89/2—Small spore-forming rods, colorless.
No. 108B/3—*Sarcina,* yellow.
No. AB—(a contaminant)—*Serratia,* red.

The purpose of this variety was to determine the effects, if any, of spore-

formers and chromogens on the growth of myxobacteria. The cells were ground lightly in an agate mortar to reduce the dried paste to a powder, and added to 1.5 per cent plain agar in the following concentrations:

00.0 milligrams per 100.0 cc. agar (control).
25.0 milligrams per 100.0 cc. agar
50.0 milligrams per 100.0 cc. agar
100.0 milligrams per 100.0 cc. agar

The first trial was with suspension 100/4, and the results of this experiment over a seven-day period are shown in Table VI. *Myxococcus fulvus* 76 and 108, *Podangium erectum* 82, *Chondrococcus blasticus* 111, and *Myxococcus xanthus* 127 were the species used for this experiment.

TABLE VI

DIAMETERS OF COLONIES IN MILLIMETERS ON VARIOUS CONCENTRATIONS OF A SPORE-FORMING BACTERIUM SEVEN DAYS—ROOM TEMPERATURE

| MGM. OF CELLS/ 100 CC. | CULT. NO. | AGE IN DAYS | | | |
|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 |
| 00.0 | 76 | 1.5 | 3.7 | 6.0 | 7.0 |
| | 82 | 1.0 | 1.5 | 2.0 | 7.0 |
| | 108 | 2.0 | 2.3 | 2.5 | 2.5 |
| | 111 | 1.5 | 2.0 | 2.0 | 2.5 |
| | 127 | 1.5 | 3.5 | 5.0 | 7.0 |
| 25.0 | 76 | 3.2 | 8.0 | 12.0 | 16.0 |
| | 82 | 2.0 | 3.0 | 5.0 | 6.0 |
| | 108 | 2.0 | 2.2 | 5.0 | 8.0 |
| | 111 | 2.0 | 7.2 | 12.0 | 18.0 |
| | 127 | ..... | 5.7 | 10.5 | 15.5 |
| 50.0 | 76 | 3.0 | 9.0 | 14.0 | 19.0 |
| | 82 | 1.5 | 1.5 | 2.0 | 12.0 |
| | 108 | 1.5 | 2.5 | 6.5 | 11.0 |
| | 111 | 2.5 | 8.5 | 15.0 | 21.0 |
| | 127 | 2.0 | 7.0 | 13.0 | 19.0 |
| 100.0 | 76 | 2.5 | 9.0 | 14.0 | 19.0 |
| | 82 | 2.0 | 3.0 | 5.0 | 11.5 |
| | 108 | 2.0 | 3.5 | 8.0 | 14.0 |
| | 111 | 2.0 | 9.5 | 16.5 | 22.0 |
| | 127 | 2.0 | 9.5 | 18.0 | 23.0 |

Examination of the data reveals a gradual but definite increase in the diameters of the colonies, in nearly every case, proportional to the increase in the cell content of the medium. There is also a corresponding tendency to produce fruiting bodies in the higher cell concentrations, while none was formed in the lower ones. It also might be noted here that with *M. fulvus* 76, *P. erectum* 82, and *M. xanthus* 127, growth actually occurred on the plain 1.5 per cent agar which acted as the control. It was thought that the distilled water used in the solutions might possibly contain sufficient minerals to support growth. Additional trials were made using glass-distilled water, but growth on those media closely approximated that shown in the above table.

Table VII gives, in condensed form, the results of growth on the three other cell suspensions previously mentioned. The incubation period was also seven days, and the plates held at room temperature. *Myxococcus fulvus* 44 was substituted for 76; all other cultures were the same.

TABLE VII

SUMMARY OF GROWTH ON VARIOUS CONCENTRATIONS OF THREE CELL SUSPENSIONS; DIAMETERS OF COLONIES IN MILLIMETERS; SEVEN DAYS—ROOM TEMPERATURE

| Cell Susp. No. | Cult. No. | Mgm. Dried Cells per 100 cc. | | | |
|---|---|---|---|---|---|
| | | 00.0 | 25.0 | 50.0 | 100.0 |
| 89/2 | 44 | 5.0 | 16.0 | 19.0 | 21.0 |
| | 82 | 2.0 | 2.5 | ..... | 28.0 |
| | 108 | 1.5 | 5.0 | 7.0 | 13.0 |
| | 111 | 7.0 | 20.0 | 23.0 | 23.0 |
| | 127 | 1.5 | 19.0 | 25.0 | 16.0 |
| 108B/3 | 44 | ..... | 17.0 | 17.0 | 25.0 |
| | 82 | ..... | 5.0 | 13.5 | 16.0 |
| | 108 | ..... | 5.5 | 11.0 | 16.0 |
| | 111 | ..... | 9.5 | 24.0 | 27.0 |
| | 127 | ..... | 14.0 | 22.0 | 29.0 |
| AB | 44 | ..... | 15.5 | 21.0 | 21.0 |
| | 82 | ..... | 2.3 | 11.5 | 15.0 |
| | 108 | ..... | 2.0 | 6.0 | 15.0 |
| | 111 | ..... | 12.0 | 26.0 | 27.0 |
| | 127 | ..... | 18.0 | 27.0 | 24.0 |

Since the various concentrations of the different suspensions were incubated at the same time, and under identical conditions, a single control was used for all. Only *Myxococcus fulvus* 44 and *Chondrococcus blasticus* 111 developed at all on the control, and then very poorly. On the three concentrations of cell suspensions the rates of growth of almost all of the organisms corresponded closely to the increase in the number of cells in the suspensions. The colonies were smallest, with one exception, on the medium containing 25 milligrams of dried cells per 100 cc. That one exception was *M. fulvus* 44 on the *Sarcina* suspension, 108B/3, and the growth rate here was the same as on the next higher concentration, i.e., 50.0 milligrams per 100 cc. Differences between growth on the 50.0 and 100.0 milligram suspensions were less marked, in many instances, some species growing less rapidly on the highest concentration. This, however, might be attributed to any one of several causes such as the size of the inoculum, which is difficult to control exactly, the manner in which it happened to be placed on the medium during the inoculation (whether it was left as a spherical fruiting body or smeared out somewhat over a larger area), etc. The fact that most of the colonies on the suspension containing 100 milligrams of cells were equal to, or larger than, those on the 50-milligram media would indicate that on the average, growth corresponded to the number of cells present.

No particular cell suspension appeared to be more favorable than any other as far as growth rates were concerned. Fruiting body production on suspension 108B/3 was somewhat better, in the highest concentration, than on the other two cell suspensions. In view of the succeeding experiment this does not appear to be significant.

The experiment was repeated using the same cell suspensions and the same species of myxobacteria. The incubation period was extended to fourteen days and the plates held at room temperature. More of the cultures produced fruiting bodies over the longer period of time, but the relationships appeared to remain the same. In every case growth on the control was poor; only *Podangium erectum* 82 produced fruiting bodies on the 00.0 concentration after fourteen days. All of the species fruited on at least one of the other three media, while *Myxococcus fulvus* 44 and *P. erectum* 82 developed fruiting bodies on every concentration of each cell suspension. There was no significant difference between the results on the three types of cells used for the suspensions, but the cell concentrations had a direct effect on rates of growth in almost every case. The difference between the two higher concentrations, 50.0 and 100.0 mgms. per 100 cc. of agar, was less marked than between the lower concentrations. It appeared that in general 50.0 milligrams of cells in 100 cc. of medium was enough to supply the needs of the myxobacteria under consideration.

In a paper read before the annual meeting of the Society of American Bacteriologists Snieszko, McAllister, and Hitchner (1941) suggested a relationship between certain species of the *Myxobacteriales* and certain gram-negative true bacteria. At about the same time that problem happened to be under consideration by the present writer. Early examination had shown most of the associated forms to be gram-negative, and the question arose as to whether or not there was a definite relationship. Since many of the soil forms are gram-negative it would be quite understandable if such were the case.

The first pair of bacteria chosen for the test, that is, to be used for the cell suspensions, was *Bacillus subtilis* and *Escherichia coli.* These were used because of both being relatively common and because either or both might possibly constitute at least a part of a natural substrate. The cells were grown in large quantities and harvested as previously described. The dried and powdered cells were added to plain agar solutions in concentrations of 100 mgm. per 150 cc. of medium. This was sterilized in the usual manner. Plates were inoculated with the following myxobacteria:

*Myxococcus fulvus* 44
*Polyangium fuscum* 77
*Podangium erectum* 82
*Chondrococcus blasticus* 111
*Myxococcus xanthus* 127

Incubation was for ten days at room temperature. Readings, i.e., measurement of the diameters of the colonies, were made at two-day intervals. The results of the six, eight and ten-day readings are shown in Table VIII, which also includes the results of a second experiment in which another pair of bacteria was employed for cell substrates. Again one gram-positive and one gram-negative species was used, but differing from the first pair in that the cells were pigmented. They were *Sarcina* sp. and *Serratia marcescens.*

TABLE VIII

COMPARISON OF SUSPENSION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA; DIAMETERS OF COLONIES IN MILLIMETERS

| AGE IN DAYS | CULT. NO. | CELLS IN SUSPENSIONS | | | |
|---|---|---|---|---|---|
| | | BACILLUS SUBTILIS GRAM + | E. COLI GRAM — | SARCINA SP. GRAM + | SERRATIA MARCESCENS GRAM — |
| 6 | 44 | 23.0 | 24.0 | 20.5 | 17.5 |
| | 77 | 9.0 | 13.0 | 6.0 | 13.0 |
| | 82 | 20.0 | 26.0 | 30.0 | 33.0 |
| | 111 | 26.0 | 27.0 | 20.0 | 26.0 |
| | 127 | 23.0 | 26.0 | 12.0 | 26.0 |
| 8 | 44 | 30.0 | 32.0 | 28.0 | 23.0 |
| | 77 | 15.0 | 19.0 | 7.0 | 24.0 |
| | 82 | 40.0 | 45.0 | 46.0 | 50.0 |
| | 111 | 34.0 | 36.0 | 25.0 | 34.0 |
| | 127 | 33.0 | 37.0 | 22.0 | 37.0 |
| 10 | 44 | 36.0 | 39.0 | 32.0 | 24.0 |
| | 77 | 17.0 | 22.0 | 7.0 | 31.0 |
| | 82 | 57.0 | 64.0 | 66.0 | 60.0 |
| | 111 | 38.0 | 42.0 | 29.0 | 36.0 |
| | 127 | 43.0 | 45.0 | 31.0 | 47.0 |

There is shown to be a slight increase in the rates of growth of all the species on the suspension of *E. coli* over that on the suspension of *B. subtilis.* However, the difference is so small that it is doubtful whether it should be considered significant. The same is true in the case of the suspensions of pigmented bacteria. The outstanding exception here is the growth of *Polyangium fuscum* 77 on the suspension of *S. marcescens;* this is much superior to that on *Sarcina sp.* However, the growth of this particular organism has been observed, in the past, to be more or less erratic, so that this difference by itself could not be considered as of very great significance. The fact that it developed more rapidly on the non-pigmented gram-negative suspension than on the gram-positive, coupled with this large difference in rate of growth on the pigmented strains might have some bearing on the matter. If there is any difference between growth of these species of myxobacteria on gram-negative and gram-positive bacterial suspensions, the data seem to point toward the gram-negative cells as the more favorable substrate.

The effect of pigmentation, too, is rather doubtful. While many of the colonies reached a somewhat greater size on the suspensions of white cells, the actual difference between rates of growth is small. Table VII showed fruiting body production to be best on suspensions of *Sarcina* while in the present case it appears to be favored by suspensions of *Bacillus subtilis*. In general, the formation of fruiting bodies parallels, more or less directly, the concentration of cells in the medium; the kind of cells used in the suspension is of less importance.

## DISCUSSION

The frequent growth on dung of the species of the *Myxobacteriales* that have been studied appears to be due, primarily, to the high bacterial content of the dung. If the water-soluble constituents of dung were necessary for growth much better development might be expected on dung decoction agar than was actually observed. This medium produced much poorer growth than even nutrient agar. The constituents utilized by the myxobacteria would obviously seem to be the water insolubles, including fecal types of bacteria. These were utilized through the agencies of bacteriolytic enzymes.

Peptone, recommended by at least one investigator as necessary for good growth, seems to have a slight, but definite, inhibitory effect when used in combination with suspension of true bacterial cells. The nature of this inhibitory action is not known, but it might affect either the myxobacterial cells themselves directly, preventing the production of enzymes, in part, or it might act on the enzyme, once it had been produced, partially or wholly destroying its activity.

In general, the kinds of associated true bacteria, acting as host cells to the myxobacteria, seem to have much less effect than the quantities in which they are present. In the absence of all other nutrient materials, with the exception of agar (which is able to support poor growth of certain species), the addition of such small amounts of dried cells as 10 milligrams per 100 cubic centimeters of medium resulted in a very definite increase in the rates of growth of all the organisms that have been studied. More than 50 milligrams of dried cells per 100 cubic centimeters of medium produced but small increases in growth rates. Under laboratory conditions this concentration of cell material seems to be optimum, rates increasing proportionally, up to this point, with the increase in concentration of cell suspension. It is possible that under natural conditions a higher concentration of host cells would be required for normal growth. The competition of the many types of soil forms would conceivably have some effect on the growth of the myxobacteria, increasing the nutrient requirements.

The gram reaction and pigmentation of the host cells may be minor factors in their utilization, though all of the myxobacterial species used in this work were able to lyse and develop upon all of the associates in

the various suspensions. It is possible that gram-negative, non-pigmented bacteria are more easily utilized by the slime bacteria, and inasmuch as such forms seem to predominate in the soil this preference could be understood. However, the myxobacteria are not especially fastidious relative to the kinds of host cells, and appear to flourish in the presence of any of the *Eubacteriales*. This relationship seems to be so definitely one of dependence that it is considered as parasitism, rather than symbiosis.

## SUMMARY

The biotic relationship of the *Myxobacteriales* and *Eubacteriales* is discussed, and it is suggested that it is one of parasitism rather than strict symbiosis or association. Cells of the true bacteria are necessary for best growth and normal development of fruiting bodies by some of the myxobacteria. The host cells are destroyed by an extracellular lytic enzyme produced by the myxobacteria.

Myxobacterial growth rates and fruiting body production increase proportionally with the concentrations of killed bacterial cells in the medium, up to about 50 milligrams per 100 cubic centimeters of medium; above that point increases in rates of growth are less marked.

The evidence indicates a possible preference on the part of the myxobacteria for gram-negative, non-spore-forming, non-chromogenic bacteria, although this is not emphasized.

## CITATIONS OF LITERATURE

BEEBE, J. M.
The morphology and physiology of certain myxobacteria of Iowa. (Abst.) Jour. Bact., 40 (1):155-156; 1940.

BERKELEY, M. J.
Intro. Crypt. Bot., 313, London, 1857.

JAHN, E.
Beitrage zur botanischen protitologie. I. Die Polyangiden. Geb. Borntträger, Leipzig; 1924.

KOFLER, L.
Die Myxobakterien der Umgebung von Wien. Sitzber. d. Kais. Akad. Wiss. Wien Math.-Nat. Klasse, 122:845-875; 1932.

KRZEMIENIEWSKI, H. AND S.
Miksobakterje Polski (Die Myxobakterien von Polen). Acta Soc. Bot. Pol., 4 (1):1-54; 1926.

MISHUSTIN, E. N.
Cellulose decomposing Myxobacteria. Microbiology (Moscow), 6(4):427-444; 1938.

PINOY, M. E.
Sur la necessite d'une association bacterienne pour la developpement d'une Myxobacterie, *Chondromyces crocatus*. C. R. Acad. Sci. Paris, 157:77-78; 1913.

QUEHL, A.
Untersuchungen über die Myxobakterien. Cent. für Bakt., II, 16:9-34; 1906.

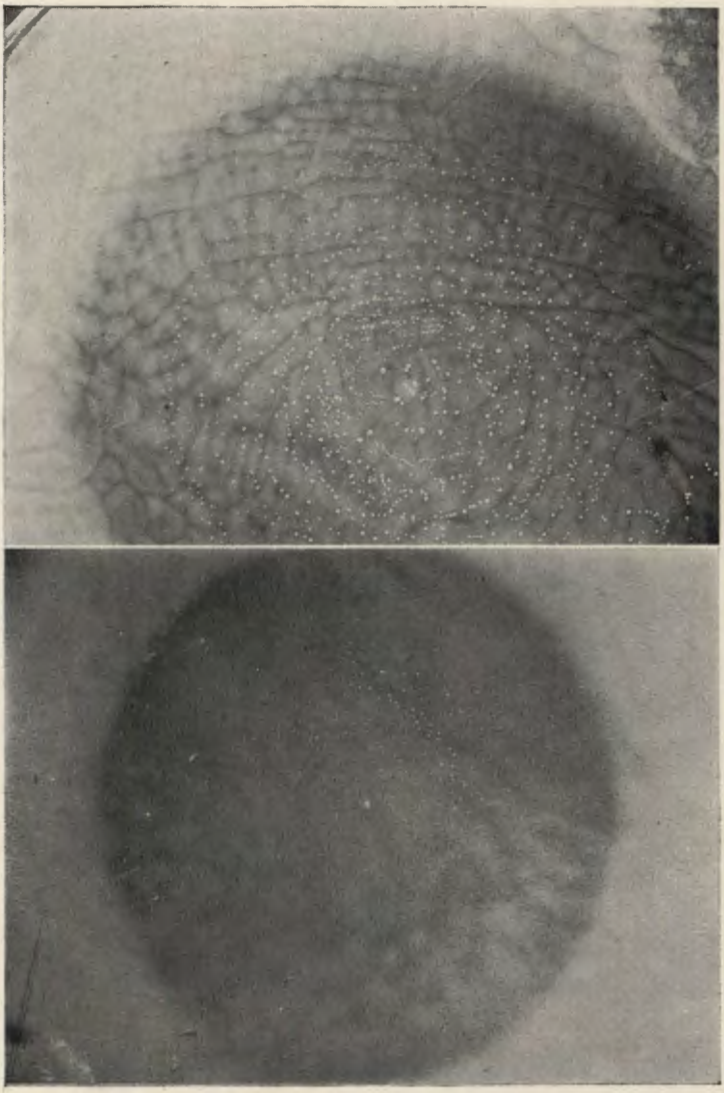
SNIESZKO, S. F., J. MCALLISTER, AND E. R. HITCHNER
On the biology of certain myxobacteria. (Abst.) Jour. Bact., 41 (1):26-27; 1941.

PLATE IV

Fig 17. 7-day-old colony of *Myxococcus fulvus* on cell suspension agar. Lysis of the cells within area of the colony may be noted. Fruiting bodies are indicated by concentric rings of white dots. About 2 X.

Fig. 18. 5-day-old colony of *Chondrococcus blasticus* on cell suspension agar showing lysis of host cells to form a translucent "window" in the opaque medium. About 2 X.

PLATE IV



# NOTE ON THE UTILIZATION OF CARBON DIOXIDE BY HETEROTROPHIC BACTERIA[1]

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Since 1935 the researches of Wood and Werkman (3, 4, 5) have shown the assimilation of carbon dioxide by heterotrophic, non-photosynthetic bacteria. In these studies species of *Propionibacterium, Escherichia,* and *Citrobacter* were used. It has been the purpose of the present investigation to determine first, the extent of carbon dioxide fixation among other heterotrophic bacteria, and secondly, the possibility of a mechanism involving carbon dioxide fixation other than the 3-carbon and 1-carbon addition made probable by Wood *et al.* (6).

In all the experiments to be reported here, cell suspensions of the various organisms were employed, with the exception of *Clostridium acetobutylicum,* which was a corn-mash culture. The substrate of *Streptococcus paracitrovorus* was 0.05 M citric acid; all other experiments contained 0.05-0.1 M glucose. Sodium bicarbonate enriched with approximately 5 per cent heavy carbon ($C^{13}$) was used in 0.05-0.125 M concentration. The fermented substrates were fractionated according to the methods employed in this laboratory, the products oxidized to carbon dioxide, and the $C^{13}$ content determined by mass spectrometer analysis. In the case of *Cl. welchii,* acetic and butyric acids were separated according to the distillation method of Schicktanz *et al.* (2). Lactic acid was degraded by $KMnO_4$ oxidation to acetaldehyde and carbon dioxide. In this reaction carbon dioxide originates from the carboxyl group and acetaldehyde from the $\alpha$ and $\beta$ carbons of lactic acid.

Reference to the table will indicate that carbon dioxide assimilation has been established with *Staphylococcus candidus, Aerobacter aerogenes, Streptococcus paracitrovorus, Clostridium welchii,* and *Clostridium acetobutylicum.* It must be remembered that naturally occurring materials contain approximately 1.09 per cent $C^{13}$. A value in excess of this figure indicates $C^{13}$ carbon is present in the compound in a concentration greater than that normally occurring. Since the bicarbonate was the only source of carbon having $C^{13}$ greater than 1.09 per cent, compounds containing a $C^{13}$ concentration greater than this value must contain fixed carbon from the bicarbonate. Experimental variation in the determinations with

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the mass spectrometer is ± 0.02. Figures indicating carbon dioxide fixation are italicized in table 1.

Lactic acid containing fixed $CO_2$ was formed by *Staph. candidus* and *Cl. welchii*. Substantially if not all, fixed $CO_2$ in this molecule lies in the carboxyl group. Acetic acid produced in the *Aerobacter* and *Cl. welchii* fermentations contained fixed carbon dioxide. Heavy carbon has been found in succinic acid in every case that it was produced, i. e., from *Staph. candidus*, *Strept. paracitrovorus* and *A. aerogenes*. All species examined have not been found to contain fixed $CO_2$ in the fermentation products. There was no indication of carbon dioxide fixation by *Lactobacillus plantarum*, *Lacto. acidophil-aerogenes* and *Strept. lactis*. It is interesting that certain compounds such as 2,3-butylene glycol and butyric acid, which are formed by synthesis, do not contain significant amounts of fixed carbon.

The fixed $CO_2$ occurring in succinic acid is believed to arise by 3 and 1-carbon addition in accordance with the Wood and Werkman reaction. With regard to the mechanism of fixation of carbon dioxide in the other products, little can be said until further information is available, particularly relative to the location of the fixed carbon atoms in the molecule. It is possible that the carbon is fixed initially by 3 and 1-carbon addition, and the final products are derived from the resulting compound. Another possibility particularly with regard to carbon dioxide fixation in the carboxyl group of lactic acid, is that of 2 and 1-carbon addition possibly involving acetylphosphate as suggested by Lipmann (1941). In this connection it is noteworthy that the lactic acid and acetic acid formed by certain bacterial species did not contain fixed carbon. This indicates a fundamental difference in the mechanism of formation of these acids among heterotrophic bacteria. The results clearly show that fixation of $CO_2$ is a general phenomenon among bacteria and has a wide application in their metabolism.

The location of fixed $CO_2$ in succinate and carboxyl group of lactic acid was shown by additional investigations with *Proteus vulgaris* to be similar to that with *Staph. candidus*.

## LITERATURE CITED

1. Lipmann, F.
1941. Advances in Enzymology, Interscience Publishers, Inc., N. Y. 1:99.

2. Schicktanz, S. T., W. I. Steele, and A. C. Blaisdell
1940. Ind. and Eng. Chem., Anal. Ed., 12:320.

3. Wood, H. G., and C. H. Werkman.
1935. Jour. Bact., 30:332.

4. ——— and ———
1938. Biochem. Jour., 32:1262.

5. ——— and ———
1940. Biochem. Jour., 34:129.

6. Wood, H. G., C. H. Werkman, A. Hemingway, and A. O. Nier
1941. Jour. Biol. Chem., 139:377.

TABLE 1

DISTRIBUTION OF FIXED CARBON DIOXIDE CONTAINING HEAVY CARBON AMONG THE FERMENTATION PRODUCTS OF HETEROTROPHIC BACTERIA

| Products | | *Staphylococcus candidus* | *Aerobacter aerogenes* | *Streptococcus paracitrovorus* | *Clostridium welchii* | *Clostridium acetobutylicum* |
|---|---|---|---|---|---|---|
| Acetic acid | m. Mols. | 54.7 | 160.0 | 137.0 | 32.3 | 34.5 |
| | % $C^{13}$ | 1.09 | *1.19* | 1.10 | *1.23* | *1.14* |
| Butyric acid | m. Mols. | | | | 16.6 | 33.9 |
| | % $C^{13}$ | | | | 1.11 | |
| Ethyl alcohol | m. Mols. | 27.6 | 64.0 | | 8.0 | 28.7 |
| | % $C^{13}$ | 1.07 | 1.10 | | 1.09 | 1.08 |
| Butyl alcohol | m. Mols. | | | | | 41.4 |
| | % $C^{13}$ | | | | | |
| Acetone | m. Mols. | | | | | 23.9 |
| | % $C^{13}$ | | | | | 1.10* |
| Lactic acid | m. Mols. | 20.0 | | 72.4 | 39.3 | |
| COOH carbon | % $C^{13}$ | *1.34* | | 1.10 (entire molecule) | *1.68* | |
| $\alpha$ and $\beta$ carbon | % $C^{13}$ | 1.11 | | | 1.13 | |
| Succinic acid | m. Mols. | 11.1 | 15.4 | 21.9 | | |
| | % $C^{13}$ | *1.25* | *1.42* | *1.31* | | |
| 2, 3-Butylene glycol | m. Mols. | | 43.0 | | | |
| | % $C^{13}$ | | 1.09 | | | |

* As 2, 4-dinitrophenylhydrazone. Yield of products expressed as m. Mols. per 100 m. Mols. substrate fermented, except *Cl. acetobutylicum* which is expressed as m. Mols. per liter. $\%\ C^{13} = \frac{\text{moles of } C^{13}}{\text{moles of } C^{13} + \text{moles of } C^{12}} \times 100$.

# A NEW GENUS AND SPECIES OF ANTHOCORIDAE (HEMIPTERA)

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Received April 30, 1941

In order to make a name available for the use of other workers, the descriptions given below have been extracted from our manuscript on the Anthocoridae in preparation and are presented at this time. The peculiar little species described occurs in nests of the wood-rat and in caves frequented by bats. The holotype (male) and allotype (female) are in the U. S. National Museum.

**Nidicola,** new genus

Small, rather oval, depressed, shiny, the head, pronotum and scutellum minutely granulose, the hemelytra very finely shagreened and with very sparse serially arranged, fine, short hairs. Head porrect, as long as broad. Eyes small. Ocelli minute, placed on a line drawn through hind margin of eyes. Antennae moderately long, segments I and II thickened, feebly pilose; III an IV very slender, clothed with long, fine hairs. Rostrum reaching between middle coxae, first visible segment reaching to a point opposite front margin of eyes; II longest, extending between anterior coxae; III slightly longer than II.

Pronotum much broader than long, the front margin slightly concave, the collar very narrow and placed entirely behind the antero-lateral angles, the disc only slightly raised, separated into two lobes by a transverse depression in front of base of scutellum, the sides very widely explanate, and somewhat reflexed, the base strongly concave. Scutellum about as long as broad, rather flat, shiny. Hemelytra rather broad, the costal areas explanate and somewhat reflexed, the clavus finely punctate, the corium also with two rows of very fine punctures. Membrane narrow, the veins obsolete, but apparently four in number. Wings undeveloped. Mesosternum small, cordate, with a fine, median longitudinal sulcus. Metasternum very small. Ostiolar canal with its rim extending outward to about middle of metapleuron, slightly curved anteriorly. Venter wide, with sparse, fine, short hairs. Legs moderate in length, with fine, short, rather indistinct hairs, the tibiae also with fine setae; tarsi three-segmented. Coxae with a row of four or five bristly hairs on antero-lateral edges, the posterior coxae approximate. Anterior femur in male fairly stout, with a row of short spinules beneath. Male genitalia asymmetrical; female with ovipositor very short.

Genotype: *Nidicola marginata,* n. sp.

Belonging to the Lyctocorinae and apparently nearest *Asthenidea* but seemingly distinct by virtue of the widely explanate pronotal and

elytral margins, the texture of the upper surface, the sparse and fine clothing, the more weakly arched rim of ostiolar canal, the short ovipositor and the armature of the front femur in the male.

**Nidicola marginata, n. sp.**

Yellowish testaceous, the head slightly darker, the eyes and ocelli reddish; shiny, appearing almost nude, the sparse, fine hairs showing only in certain lights. Head measured to collum as broad as long, (16:16), sharply narrowed in front of eyes, then about parallel-sided along apical third. Vertex arched. Eyes small, oblong-oval as seen from above; circular, from the side. Ocelli minute, sometimes indistinct. Antennae slender, segment I not attaining apex of head, II distinctly enlarged from the base, III and IV slender, the clothing hairs on IV longer than on others; proportions, 5:12:11:12. Rostrum long; proportions, 8:16:11.

Pronotum twice as wide as long (32:16), the anterior angles produced well in front of the very narrow collar, the disc slightly raised, the transverse impression interrupted on each side. Hemelytra widened, rather translucent, the punctures and clothing hairs indistinct in certain lights. Venter of male with a stout seta on each side of midline of third segment, the line between these setae carinate and ciliate. Sixth segment of venter of female sinuate at apex and slightly produced at mid-line over base of the short ovipositor. Left clasper of male tapering to a fine point, somewhat curved and sinuate, its length about equal to transocular width.

*Length:* 1.90—2.10 mm. *Width:* pronotum, 0.73 mm.; abdomen, 1.00 mm.

*Holotype,* male, and *allotype,* female, Picacho Pk., Arizona, Nov. 3, 1940, R. A. Flock (in collection of U. S. National Museum). *Paratypes,* twelve males and females taken in bat caves with types; nine males and females, Tucson, Arizona, H. G. Hubbard, taken in nest of wood-rat *(Neotoma albigula)*; one male, Ft. Yuma, Arizona, H. G. Hubbard; one male and one female, Palm Springs, California, Hubbard; and one female, Indio, California, May 24, 1934, F. S. Stickney, in decaying dates (in collections of U. S. Nat. Mus., Univ. of Arizona, R. C. Flock, and authors).

The specimens taken by Hubbard bear manuscript names of Uhler, labeled as new genus and species. Fully winged individuals are desired as the unique pronotal characters may be associated in part with the partial failure of wing development.

The Board of Editors of the Iowa State College Journal of Science dedicates this issue to the memory of Percy Edgar Brown, business manager of this Journal from 1926 through 1936, and Editor-in-Chief in 1936-37.

The Board also wishes to thank Dr. A. G. Norman and the staff of the Department of Soils for their co-operation in collecting and preparing the material presented in this number of the Journal.

# *Percy Edgar Brown*

## *1885-1937*

Agriculture and the related sciences are deeply indebted to many men who, like Percy Edgar Brown, worked diligently to discover new knowledge and train young men to "understand and to use both new and existing knowledge for the benefit of mankind."

"A man's greatest privilege is to exercise an influence which will go on after he is through work." It certainly can be stated with emphasis that Doctor Brown's influence in the fields of Soil Bacteriology, Soil Fertility, and Soil Survey will be a potent factor through the years to come, in inspiring and helping men to strive for a broader knowledge of soils and of the soils problems which must be solved if the United States and other nations are to have a profitable and enduring agriculture.

Doctor Brown was recognized as an authority in this country and abroad. He was the author of an unusually large number of scientific articles, bulletins, and special reports; he was an administrator of outstanding ability, and rendered distinguished service to agriculture and science as an officer in various state and national organizations. For a number of years he was secretary of Section "O" (Agriculture) of the American Association for the Advancement of Science, and a fellow of that Association, a fellow of the Iowa Academy of Science, and a member of the American Chemical Society. In 1920 he was elected secretary-treasurer of the American Society of Agronomy, a position he held until the time of his death except for the year 1932 when he served as president. He was elected a fellow of the Society in 1925. Doctor Brown's great contribution to that organization over a long period of years has been referred to by the editor of the Journal of the American Society of Agronomy in these words: "I feel that the American Society of Agronomy and all that it stands for today is one of the many splendid monuments that Doctor Brown has left to his enduring memory."

In 1926 he was president of the American Soil Survey Association; in 1913, Councilor for the Society of American Bacteriologists; in 1918, expert on the National Research Council; during the period 1926-36 he was Business Manager of the Iowa State College Journal of Science. He was Editor-in-Chief of the Journal at the time of his death. He was also a member of the American Organizing Committee for the First International Congress of Soil Science, and one of the twelve delegates appointed to attend that convention in Washington.

But Doctor Brown did more than these things; he had the courage and vision to insist that members of his staff and his

students maintain at all times the high ideals of scholarship and responsibility which to him were of the utmost importance. He believed in these ideals and practiced them in his daily life.

It is not surprising that a man with such a background established an enviable record as a leading educator, research worker, administrator, and author. Although a relatively young man when he finished his work, Doctor Brown left a long list of publications based for the most part on his extensive studies and researches in soil bacteriology, soil fertility, and soil management. Many of his journal articles and bulletins are notable because they make available to students of soil science new and worth-while data which will contribute in no small measure to the solution of many problems in the fields of agriculture and soil bacteriology.

W. H. STEVENSON

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