

STRONGYLOCORIS BLANCHARD: SIX NEW SPECIES FROM NORTH AMERICA (HEMIPTERA, MIRIDAE)

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Only six species of *Strongylocoris* have heretofore been recognized from North America, but the present paper raises the total number to twelve. Eight species and certain varieties are recorded from the Palearctic region, making a total of twenty species known for the world.

This genus of Mirid bugs is composed chiefly of black, shining species, all of which have a rather similar aspect. This similarity of form has made the determination of species difficult, except when reference is made to structure of the male genitalia. The present study of the male claspers has enabled the author to separate the species without difficulty, except in the case of certain female specimens. Slight variations have been found in the number of spines on a clasper as shown for *S. stygicus* Say (Plate 1, fig. 1), but the general form of both claspers, when considered together, give distinctive characters for the separation of the species.

Strongylocoris breviatus new species

Allied to *stygicus* Say but distinguished by the yellowish first and second antennal segments, the latter with distinct black band at base; male genital claspers distinctive of the species (Plate 1, fig. 3).

Male. Length 4.3 mm., width 2 mm. Head: width 1.06 mm., vertex .56 mm. Rostrum, length 1.05 mm., just reaching to base of middle coxae, black. Antennae: segment I, length .35 mm., yellowish brown, blackish on base; II, 1.3 mm., brownish to black, brownish on basal half and black on base; III, .95 mm., blackish; IV, .47 mm., blackish. Pronotum: length .91 mm., width at base 1.51 mm. Hemelytra with costal margin moderately arcuate. Dorsum finely and closely rugulose punctate, sparsely clothed with short pale pubescence, more apparent on lateral margins of hemelytra, the paracuneus with three or four long hairs.

General coloration deep black, shining; femora black, apices yellowish, tibiae yellowish, apices and more or less on basal half of hind pair fuscous; tarsi yellowish, the apical segment black.

Female. Length 4.1 mm., width 2.2 mm.; hemelytra more sharply arcuate than in the male. Head: width 1.2 mm., vertex .64 mm. Antennae: segment I, length .36 mm., yellow, base black; II, 1.17 mm., yellow, apical one-fourth and narrow ring at base, blackish; III, .86 mm., black, yellowish at base; IV, .44 mm., blackish. Coloration of the second antennal segment distinctive of the species.

Holotype: ♂ July 2, 1926, Washington, D. C. (H. H. Knight); author's collection. *Allotype*: same data as the type. *Paratypes*: 23 ♂ ♀, taken with the type on *Solidago* sp. DISTRICT OF COLUMBIA—7 ♂ ♀ June 24, 3 ♂ June 30, 2 ♂ July 8, 1926, Washington (H. H. Knight). ILLINOIS ♂ 2 ♀ July 17, 1883, Anna (C. A. Hart) ♂ July 5, 1932, Antioch (Frison

et al). ♀ Aug. 25, 1925, Beech (T. H. Frison). 27 ♂ ♀ June 25, 1932, Browns, on Solidago (Ross, Dozier, Park). ♂ June 12, 1933, Bureau (Mohr and Townsend). 2 ♂ 4 ♀ July 2, 1932, Castle Rock, Grand Detour (Dozier and Mohr). ♂ June 5, 1889, Champaign (C. A. Hart). 2 ♀ June 14, 1933, Dolson (Frison and Ross). 22 ♂ ♀ June 30, 1932, Galens (Dozier and Mohr). ♂ June 22, 1932, Golconda (Ross and Dozier). ♀ June 24, 1932, Herod (Ross, Dozier, Park). ♂ May 21, 1932, Makanda (Dozier). ♂ June 14, 1933, Marshall (Frison and Ross). ♂ July 2, 1906, Mt. Carmel (C. A. Hart). 3 ♂ 2 ♀ June 14, 1930, Oakwood (Frison). ♀ July 9, 1925, Oregon (Frison). 2 ♀ May 29, 1907, Pulaski (Hart). ♂ June 25, 1932, Shawneetown (Ross and Dozier). ♂ June 9, 1885, Urbana (C. A. Hart), on Helianthus. 2 ♀ June 26, 1932, West Union (Ross and Dozier). MARYLAND—♀ July 17, 1926, Glen Echo (H. H. Knight). MASSACHUSETTS—♂ ♀ July 7, 1928, Sherborn (C. A. Frost). MAINE—2 ♀ July 10, Muddybemos; ♀ June 15, 1922, Orono (R. J. Sim). MINNESOTA—♂ July 2, 1923, St. Anthony Park; ♀ Aug. 10, 1922, Cramer; 12 ♂ ♀ Aug. 15, 1925, Isabella River, Lake Co.; 6 ♂ ♀ Aug. 20, 1920, Two Harbors (H. H. Knight). ♀ June 20, 1923, Norman Co.; 2 ♂ July 21, 1922, Minnetonka Lake; ♀ Aug. 2, 1921, Cloquet (A. A. Nichol). ♂ June 28, 1921, Hennepin Co. (A. T. Hertig). ♀ July 19, 1921, Princeton (W. E. Hoffmann). MONTANA—♂ July 23, 1912, Bozeman. NEW YORK—27 ♂ ♀ July 8, 1920, Ithaca (H. H. Knight), taken on *Solidago altissima* with nymphs. ♂ July 24, ♂ July 26, 1916, Ithaca (H. H. Knight). ♂ June 28, ♂ July 26, 1914, 2 ♂ Aug. 5, ♂ Aug. 12, ♂ Aug. 13, ♀ Aug. 19, 1915, Batavia (H. H. Knight), on Solidago. ♂ ♀ July 27, 1916, McLean (H. H. Knight). NEW HAMPSHIRE—♂ June 30, 1929, Hampton (S. A. Shaw). PENNSYLVANIA—♂ June 28, 1919, Hartstown (D. M. Delong). WYOMING—♂ Aug. 17, 1927, Ten Sleep (H. H. Knight). CANADA—ALBERTA: 9 ♂ ♀ Aug. 17, 1924, Slave Lake (O. Bryant). NOVA SCOTIA: 13 ♂ ♀ July 30, 1929, Portauapique (C. A. Frost).

Strongylocoris atritibialis new species

Distinguished from *stygicus* Say by the more arcuate hemelytra, black tibiae, and in structure of the male genital claspers.

Male. Length 4.6 mm., width 2.4 mm. Head: width 1.21 mm., vertex .65 mm. Rostrum, length 1.12 mm., reaching to base of middle coxae. Antennae: segment I, length .34 mm.; II, 1.26 mm.; III, 1.00 mm.; IV, .43 mm.; black. Pronotum: length 1.12 mm., width at base 1.77 mm. Hemelytra with costal margin strongly arcuate. Dorsal surface nearly glabrous, rugulose punctate, sparsely clothed with fine, short pubescent hairs, more evident on cuneus and embolium. General coloration deep black, membrane brownish black; legs black, tibiae and tarsi sometimes brownish black, but hind tibiae always black. Genital claspers distinctive of the species (Plate 1, fig. 6).

Female. Length 4.5 mm., width 2.5 mm.; embolar margins more strongly arcuate than in the male. Head: width 1.3 mm., vertex .78 mm. Antennae: segment I, length .35 mm.; II, 1.21 mm.; III, .95 mm.; IV, .40 mm. Pronotum: length 1.12 mm., width at base 1.86 mm. More robust than the male but very similar in coloration.

Holotype: ♂ June 23, 1920, Ithaca, New York; author's collection. *Allotype*: same data as the type. *Paratypes*: ♂ ♀, taken with the types.

COLORADO—4 ♂ 5 ♀ August 11, 1925, Ute Creek Ranch, Fort Garland (H. H. Knight). ♂ June 12, ♂ June 28, 1900, Fort Collins; 8 ♂ ♀ August 19, 1898, Dixon's Canyon, Fort Collins (E. D. Ball). CONNECTICUT—♀ June 27, 1920, Kinningworth (W. E. Britton). ♀ June 22, 1920, Orange (P. Garman). DISTRICT OF COLUMBIA—10 ♂ ♀ June 24, ♀ June 30, 2 ♀ July 2, 1926, Washington (H. H. Knight). ILLINOIS—♂ July 7, 1909, Algonquin (Nason). 8 ♂ 10 ♀ June 10, 1932, Antioch (Mohr and Townsend). 3 ♂ 8 ♀ July 5-7, 1932, Antioch (Frison et al), 2 ♀ June 12, 1936, Antioch (Ross and Burke). ♂ June 25, 1932, Browns (Ross, Dozier and Park). ♀ July 2, 1932, Castle Rock, Grand Detour (Dozier and Mohr). ♂ June 8, 1931, Charleston (Frison). 5 ♂ 2 ♀ May 17, 1932 (H. L. Dozier). ♂ May 22, 1916, ♂ 5 ♀ May 12, 1916, Dongola (C. A. Hart). ♀ June 25, 1932, Rocky Branch, Dolson (Frison and Mohr). ♂ July 6, 1917 (C. A. Hart); ♂ May 27-31, 1932 (H. L. Dozier). ♂ May 15, 1932, Fountain Bluff (Frison, Ross and Mohr). 2 ♂ 2 ♀ June 30, 1933, Galena (Dozier and Mohr). ♀ June 28, 1893, 2 ♂ July 7, 1892, Galesburg (Stromberg). ♂ June 10, 1936, Grays Lake (Ross and Burke). ♀ May 31, 1933, Havana (C. A. Mohr). ♂ May 29, 1936, Herod (Ross and Mohr). ♂ ♀ June 9, 1933, Joliet (Mohr and Townsend). 2 ♀ May, 1932, Makanda (Dozier and Mohr). ♂ May 24, 1914, Muncie (C. A. Hart). ♂ ♀ May 25, 1932, Pulaski (H. L. Dozier). ♀ May 27, 1928, Shawneetown (T. H. Frison). 2 ♀ June 4, 1932, Sheldon (Frison and Mohr). 2 ♂ June 4, 1932, St. Anne (Frison and Mord). 2 ♀ June 11, 1936 (Ross and Burke); 2 ♀ July 15, 1926 (T. H. Frison). IOWA—16 ♂ ♀ June 4, 1926, 2 ♀ June 9, 1925, 20 ♂ ♀ June 18, 1925, Ames (H. H. Knight). KENTUCKY—♀ May 12, 1921, Lexington (K. S. B.). MASSACHUSETTS—♀ July 7, 1928, Sherborn (C. A. Frost). MINNESOTA—♂ June 12, 1922, Faribault; ♂ June 25, 1921, Madison; ♂ 2 ♀ June 28, 1921, Olivia; 3 ♂ 5 ♀ July 2, 1923, St. Anthony Park (H. H. Knight). ♂ June 18, 1922 Minneapolis; ♂ June 25, 1922, Ramsey Co.; ♂ ♀ June 28, 1921, Hennepin Co. (A. T. Hertig). ♂ ♀ July 10, 1920, Morrison Co.; ♀ June 21, ♂ June 24, 1921, Owatonna (A. A. Nichol). MISSOURI—♂ 2 ♀ May 16, ♂ May 23, Glencoe; ♀ June 19, 1937, St. Louis (R. C. Froeschner). NEW JERSEY—♂ ♀ June 2, 1925, Campgaw (F. M. Schott). NEW YORK—♀ June 14, 1915, ♂ July 12, 1914, Batavia; ♀ July 7, 1920, Ithaca; ♂ June 27, 1915, Portage; ♂ July 3, 1920, McLean Bogs, Tompkins Co. (H. H. Knight). ♀ June 14, 1919, White Plains (J. R. Torre-Bueno). NORTH CAROLINA—♂ June, 1907, Hendersonville (F. Sherman). ♀ June 200, 1916, Swannanoa (R. W. Leiby). NORTH DAKOTA—♂ 2 ♀ July 14, 1920, Kidder Co. (A. A. Nichol). SOUTH DAKOTA—♀ July 29, 1927, Deadwood (H. H. Knight). ♂ ♀ June 12, 1923, Brookings; ♂ June 23, 1923, Philip (H. C. Severin). OHIO—♂ July 17, 1926, Wilmington (S. A. Watson). PENNSYLVANIA—7 ♂ ♀ June 8, 1921, 6 ♂ ♀ June 7, ♀ June 8, 1923, Chambersburg (J. R. Stear). 2 ♀ June 17, 1917, Charter Oak (J. N. Knull). TENNESSEE—♀ May 15, 1917, Knoxville, (Geo. G. Ainslie). WASHINGTON—2 ♂ 1 ♀ July 6, 1937, Dayton (R. E. Miller). WYOMING—♀ July 16, 1920, Yellowstone National Park (A. A. Nichol). 46 ♂ ♀ August 7, 1927, Shoshone National Forest (H. H. Knight). CANADA—ALBERTA: ♂ July 31, 1921, ♀ July 29, ♂ August 6, Nordegg (J. McDonnough). ONTARIO: 4 ♂ ♀ July 10, 1915, Parry Sound (H. S. Parish).

***Strongylocoris pallidicornis* new species**

Allied to *atritibialis* as indicated by the structure of the male genital claspers but differs in the longer second antennal segment which greatly exceeds the width of head across eyes; first and second antennal segment and all three pairs of tibiae, pale.

Male. Length 4.55 mm., width 1.9 mm. Head: width 1.08 mm., vertex .56 mm. Rostrum, length 1.12 mm., reaching to middle of intermediate coxae, blackish, middle one-third yellowish. Antennae: segment I, length .34 mm., yellowish, fuscous on base; II, 1.25 mm., pale to yellowish, frequently fuscous on apical one-fifth; III, .91 mm., blackish, narrowly pale at base; IV, .47 mm., fuscous. Pronotum: length .91 mm., width at base 1.55 mm. Hemelytra with costal margin only very slightly arcuate. Dorsum sparsely clothed with moderately short, brownish pubescent hairs, more prominent on embolium and lateral margins of pronotum. General coloration black, shining, dorsum rather thickly rugulose punctate; membrane brownish black, paler apically. Legs black, apices of femora and coxae, tibiae, and tarsi except apical segment, pale to yellowish. Genital claspers rather similar to those of *atritibialis*, but terminal portion of right clasper somewhat different (fig. 5).

Female. Length 4.2 mm., width 2.2 mm.; embolar margins more arcuate than in the male, but not so strongly arcuate as in *atritibialis*; antennae and legs more broadly pale than in the male. Head: width 1.12 mm., vertex .62 mm. Antennae: segment I, length .35 mm., pale yellowish; II, 1.12 mm., pale yellowish, slightly fuscous on apex; III, .82 mm., fuscous, narrowly pale at base; IV, .39 mm., fuscous. Pronotum: length .91 mm., width at base 1.6 mm.

Holotype: ♂ August 19, 1927, Custer, South Dakota (H. H. Knight); author's collection. *Allotype*: July 27, 1927, Custer, South Dakota (H. H. Knight). *Paratypes*: 1 ♂ 1 ♀ July 27, 3 ♂ 3 ♀ August 19, 1927, Custer, South Dakota (H. H. Knight). COLORADO—3 ♂ 1 ♀ August 7, 1925, Stonewall, alt. 8,500 ft., near Trinidad (H. H. Knight). ♂ August 11, 1925, Ute Creek Ranch, Fort Garland (H. H. Knight). 12 ♂ 4 ♀ August 19, 1898, Dixon's Canyon, Fort Collins (E. D. Ball). WYOMING—3 ♂ 4 ♀ July 20, 1920, Yellowstone National Park (A. A. Nichol). NORTH DAKOTA—2 ♀ July 19, ♀ August 4, 1923, Trail Co. (A. A. Nichol). ♂ July 12, 1920, Cass Co. (A. A. Nichol). MINNESOTA—♂ June 20, 1921, New Ulm (H. H. Knight). ♂ June 21, ♀ June 25, ♂ July 20, 1923, Norman Co. (A. A. Nichol). 3 ♀ July 25, 1921, Eagle Bend (A. A. Nichol).

***Strongylocoris hirtus* new species**

Distinguished from allied species with erect pubescence by the longer second antennal segment which exceeds width of head across eyes; tibiae and first antennal segment pale; male genital claspers distinctive (Plate 1, fig. 4).

Male. Length 4.6 mm., width 2.2 mm. Head: width 1.12 mm., vertex .56 mm. Rostrum, length 1.12 mm., just reaching to base of middle coxae; black, third segment except base and basal half of fourth segment, pale. Antennae: segment I, length .38 mm., pale, base fuscous; II, 1.43 mm., black, basal one-fifth pale; III, .86 mm., black; IV, .47 mm., black. Pronotum: length .98 mm., width at base .165 mm. Hemelytra with costal margin

moderately arcuate on distal half. Clothed with thickly set, erect, golden brown to blackish pubescence.

General coloration black, slightly shining; legs black, tibiae, tarsi except apical segment, and apices of femora, pale, tibial spines fuscous.

Female. Length 4.3 mm., width 2.4 mm. Head: width 1.12 mm., vertex .60 mm. Antennae: segment I, length .35 mm.; II, 1.21 mm., pale, apical one-fourth blackish; III, .74 mm.; IV, .43 mm. More robust than the male but very similar in color and pubescence.

Holotype: ♂ July 1, 1929, Ames, Iowa (H. H. Knight); author's collection. *Allotype*: taken with the type. *Paratypes*: 39 ♂ ♀, taken with the types on *Silphium perfoliatum* L. which is the host plant. 12 ♂ ♀ July 13, 27 ♂ ♀ July 18, 1927, 47 ♂ ♀ June 21, 1928, 8 ♂ ♀ July 11, 1929, 54 ♂ ♀ July 2, 1930, ♂ 3 ♀ June 24, 1931, Ames, Iowa (H. H. Knight), all collected on *Silphium perfoliatum*. ♂ 3 ♀ July 5, 1927, Ames Iowa (H. G. Johnston). ILLINOIS—♂ June 14, 1931, Charleston (H. H. Ross). ♂ June 5, 1932, Hardin (H. L. Dozier). 4 ♂ 1 ♀ June 14, 1930 (T. H. Frison), on *Silphium*. ♂ ♀ June 26, 1932, West Union (Ross and Dozier). KANSAS—♂ July, Topeka (Popenoe).

Strongylocoris ambrosiae new species

Allied to *hirtus* but readily distinguished by the smaller size, black antennae with shorter second segment, and in structure of the male genital claspers.

Male. Length 3.7 mm., width 1.5 mm. Head: width .92 mm., vertex .48 mm. Rostrum, length .87 mm., reaching upon base of middle coxae. Antennae: segment I, length .23 mm.; II, .82 mm., not equal to width of head; III, .60 mm.; IV, .30 mm. Pronotum: length .69 mm., width at base 1.3 mm. Hemelytra with costal margin only very slightly arcuate. Dorsum thickly clothed with erect, brownish black pubescent hairs. General coloration black, moderately shining; tibiae and tips of femora pale to yellowish, tarsi fuscous to black, scarcely paler on base. Genital claspers distinctive of the species (fig. 2).

Female. Length 3.5 mm., width 1.8 mm. Head: width .99 mm., vertex .56 mm. Antennae: segment I, length .22 mm.; II, .92 mm.; III, .65 mm.; IV, .30 mm. Pronotum: length .78 mm., width at base 1.4 mm. Hemelytra with costal margin more arcuate but very similar to the male in color and pubescence.

Holotype: male, August 24, 1927, Emery, South Dakota (H. H. Knight); author's collection. *Allotype*: taken with the type. *Paratypes*: 46 ♂ ♀, taken with the types on *Ambrosia psilostachya*. IOWA—♀ May 22, 1936, Ames (G. Bleasdel). 2 ♂ May 29, 1936, Ames (H. H. Knight). TEXAS—2 ♂ 3 ♀ September 28, 1930, Amarillo (S. E. Jones). KANSAS—♀ July 6 (Popenoe); ♀ July 2, 10 ♂ ♀ August 17, ♀ August 18, Riley Co. (G. A. Dean). 4 ♂ ♀ August 31, Wallace Co.

Strongylocoris albibasis new species

Distinguished from known species by the small size and white base of vertex; male genital claspers distinctive of the species (Plate 1, fig. 8).

Male. Length 3.3 mm., width 1.6 mm. Head: width .82 mm., vertex .4 mm.; basal edge of vertex bluntly carinate, white. Rostrum, length .86

mm., extending to tip of intermediate coxae. Antennae: segment I, length .22 mm., black, tip pale; II, .90 mm., black; III, .61 mm., black; IV, .35 mm., black. Pronotum: length .69 mm., width at base 1.25 mm. Hemelytra with costal margin only slightly arcuate. Dorsum finely rugulose punctate, shining, sparsely clothed with short pale pubescence.

General coloration shining black, base of vertex and posterior margins of eye, white; legs black, tips of femora pale, tibiae pale, hind pair black, sometimes slightly paler on apical half; tarsi black, scarcely paler at base.

Female. Length 3 mm., width 1.7 mm.; hemelytra with costal margin rather strongly arcuate. Head: width .86 mm., vertex .47 mm.; base of head white as in the male. Antennae: segment I, length .21 mm.; II, .78 mm.; III, .56 mm.; IV, .30 mm. More robust than the male but very similar in color and pubescence.

Holotype: ♂ June 2, 1926, alt. 4,500 ft., Tombstone, Arizona (A. A. Nichol); author's collection. *Allotype*: same data as the type. *Paratypes*: 4 ♂ 1 ♀, taken with the types.

PLATE I

Explanation of figures

Male genital claspers of *Strongylocoris*. a. left clasper, postero-lateral aspect. b. right clasper, postero-lateral aspect. b'. variation in apex of right clasper.

Fig. 1. *Strongylocoris stygicus* Say.

Fig. 2. *Strongylocoris ambrosiae* n. sp.

Fig. 3. *Strongylocoris breviatus* n. sp.

Fig. 4. *Strongylocoris hirtus* n. sp.

Fig. 5. *Strongylocoris pallidicornis* n. sp.

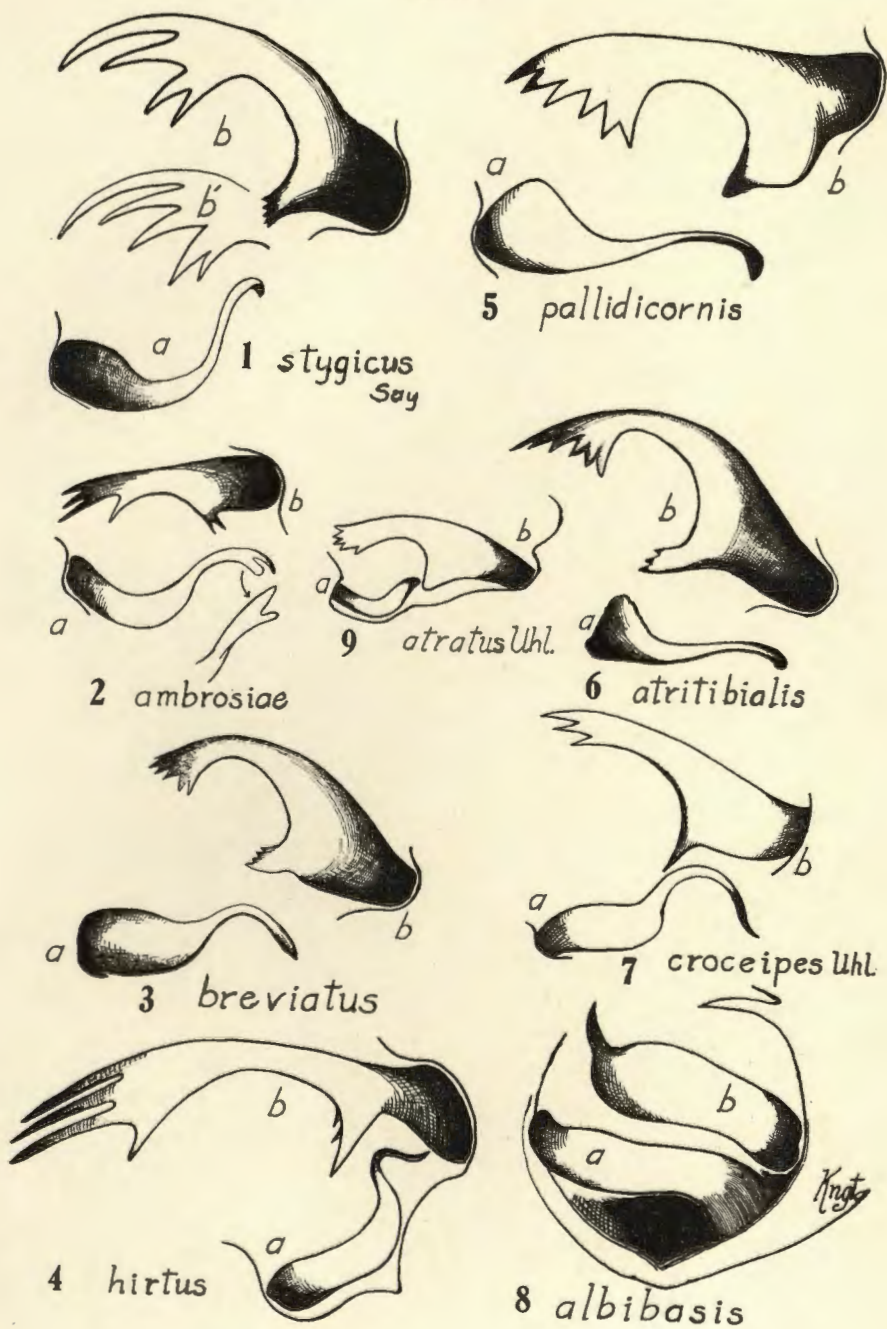
Fig. 6. *Strongylocoris atritibialis* n. sp.

Fig. 7. *Strongylocoris croceipes* Uhl.

Fig. 8. *Strongylocoris albibasis* n. sp.

Fig. 9. *Strongylocoris atratus* Uhler, drawn on reduced scale, from the type in the California Academy of Sciences.

PLATE I



THE FORMATION OF VITAMINS A, B₁, AND C IN LEMNA GROWN IN THE ABSENCE OF ORGANIC MATTER

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The effect of organic material on growth and reproduction of green plants has been studied for many years. From early work on the amount of crops produced, to recent investigations on plant hormones, the subject has received constant attention, but comparatively little work has been done on the effect of organic matter on the formation of vitamins in the plant, or on conditions which may affect the quality of crops in this respect.

The question of the effect of organic matter on the vitamin content of grains was first raised in India, when an investigation was made to determine if the quantity of vitamin in plants was increased by the presence and availability of organic matter in the soil. This question is of major importance to a country in which a large proportion of the food is derived from grains, especially in view of the increasing use of inorganic fertilizers, and is of considerable interest to investigators of vitamins and of agricultural products.

McCarrison (17) in southern India found the quantity of vitamins in grains (particularly the B complex and to some extent vitamin A also) varied considerably. The variation seemed to be correlated with the organic matter available to the plant. Viswa Nath (21) came to the conclusion either that bacteria synthesized vitamins from organic matter and passed them on to the plant, or that the microorganisms produced substances which stimulated the formation of vitamins within the plant. On the other hand, at the Ohio Experimental Station, the findings of Hunt (15) seem to indicate no correlation between the presence of the vitamin and the supply of organic matter. He checked the amount of the vitamin B complex in wheat grown on plots that had been treated for 35 years with different kinds of fertilizers, and found little influence due to the treatments. A variation occurred, but it seemed to be attributable more to climate than to fertilizers or organic matter.

A somewhat similar theory to that of Viswa Nath's had been suggested by Bottomley and Mockeridge fifteen years earlier. Bottomley (4), soon after the essential nature of vitamins in animal life was recognized, introduced the idea of plant vitamins or auximones. According to this theory it was necessary to supply the organic auximones in order to obtain normal functioning of the plant, and Mockeridge (18) concluded that the more bacterial decomposition there was in organic manures, the more auximone was formed.

The claim that these organic substances were essential for the growth and reproduction of all green plants was shown by Clark and Roller (8)

¹ Part of this investigation was made possible by a grant from the Rockefeller Fluid Research Fund of Iowa State College.

to be without foundation, and this was confirmed by Saeger (19) and other investigators. Clark and Roller showed that the green plant *Lemna* would reproduce in inorganic solutions, in the absence of both organic matter and microorganisms. However, in some cases there were definite effects on growth and reproduction of *Lemna* from the addition of organic matter to the nutrient medium in which the plant was growing, especially in the presence of bacteria and other microorganisms.

Recently it has been demonstrated that plants themselves produce certain organic substances (auxins) which are essential for their life processes (16, 22). These may behave as plant hormones, being produced and transported in the plant itself, as is the case with hormones in the animal body. The auxins were at first believed to be few in number and specific in their action, but further investigation disclosed many substances which acted in a similar way (14). Further, when extracted auxins, or similar synthetic compounds, were supplied to the plant—for example, by injection, or by means of the soil or nutrient solution—increased growth or reproduction occurred, causing curvature of coleoptiles, bending of plant stems, or growth of adventitious roots.

It seemed logical that substances like the vitamins, which occurred so frequently in plants, should have some specific function in plant life. From one point of view, the value of these vitamins is that they are essential to animals, but plants would not produce them for that purpose, although animals may have evolved so as to make use of the vitamins produced. A number of the vitamins have been tested as plant growth-promoting substances and found to have a definite effect on growth. For example, van Hausen (12) reported that the addition of vitamin C to pea cultures caused an increase in dry weight, and exerted a marked influence on young plants. Bonner (3) came to the conclusion that vitamin B₁ was as necessary for plant growth as it was for the normal growth of animals.

Bonner suggests that all the various organic substances which have definite beneficial effects on plant growth or reproduction should be classed as 'plant vitamins'. This would include the auxins or plant hormones, some or all of the animal vitamins, the 'bioses' of yeast, 'pantothenic' acid and others. He also suggests that all plants may be classified as heterotrophic or autotrophic. To the first group, plant vitamins must be supplied; to the second, no organic material need be given, as the plant synthesizes its own. How many green plants are completely autotrophic is not known.

Lemna major seems to be definitely autotrophic. It has been used by one of the authors (N. A. C.) for several years in investigations on the effect of organic matter on reproduction and growth (6). This plant can produce flowers, but it usually buds asexually. It has been grown successfully, not only in soil solutions, but in entirely inorganic media, with and without microorganisms, and under artificial light, as well as in sunlight. The *Lemna* has passed through hundreds of generations, quite free from organic matter or bacteria, both in the media and in the plant itself. It was therefore possible to determine whether a plant so produced would contain any vitamin at all, and to make some attempt at comparison with the content of similar plants grown under more normal conditions.

VITAMIN A

Four groups of rats were given a diet deficient in vitamin A until their weights either remained stationary for several days or began to decrease markedly. Frequently the early symptoms characteristic of vitamin A deficiency were observed. After satisfactory depletion, three of the groups were fed Lemna as a supplement, while the fourth group served as a control. When the basal ration was supplemented with Lemna grown in a soil-water medium (with ample organic matter available and with no attempt to control microorganisms), the deficiency in the diet was corrected. The xerophthalmia cleared up rapidly and the rats regained health and added weight. Lemna grown in inorganic nutrient media (specially purified salt solutions, in the absence of all microorganisms), and through a long series of generations, was fed in the same way and was equally potent in meeting the deficiency. Details of these experiments were published earlier (6, 7).

VITAMIN B₁

The first attempt to find vitamin B₁ in the Lemna was made with *Drosophila melanogaster*, as the B complex had been reported essential in the life cycle of the fly, and considerably less material would be required than for rat feeding. The attempt, however, was not successful. If it had been feasible to produce the sterile larvae on synthetic media, adding the Lemna or its extract to provide the vitamin, a considerable amount of time would have been saved. To obtain a supply of Lemna, free from microorganisms and sufficient to feed groups of rats, necessitates drying and storage of the plants, and this treatment has some influence on the quantity of the vitamin, as noted in the experiments with vitamin A (7).

As the experiments with the *Drosophila* were unsuccessful, it was necessary to prepare the larger quantities required for rats. One lot of plants was grown in the absence of microorganisms, in the inorganic culture medium, with special precautions to exclude all organic matter. The plants were produced in Erlenmeyer flasks with cotton wool stoppers, collected once a week after checking for contamination, washed, dried and kept in the dark. These plants received light from Mazda lamps only, with the temperature held at 25°C. The non-sterile Lemna were produced in Erlenmeyer flasks or in large dishes in a soil-water mixture. These received sunlight, supplemented in winter with some artificial light. The plants were collected and dried in the same way as the others.

Two series of tests were run, about a year apart. Some vitamin B₁ was expected in the Lemna, but it was impossible to estimate how much. The first series confirmed the expectation and indicated the approximate quantity.

SERIES 1

This series included 55 rats in groups, with litter mates separated in different groups. All were fed the Chase and Sherman (5) basal ration, diet 513, without B₁, until the body weights had dropped appreciably. As a positive control, 4 rats were then fed 200 mg. each per day non-autoclaved yeast. Recovery was rapid and growth continued until shortly after the yeast was removed from the ration (fig. 1, curve 7).

The negative control groups (fig. 1, curves 1 and 1a), consisted of 12 rats. These received as much as they would eat of the basal diet and 500 mg. daily per rat of autoclaved yeast in which B_1 had been destroyed. Six of these rats died before the end of the experiment and two others shortly afterwards (fig. 1, curve 1). Each of the four remaining rats was given 50 mg. daily of the B_1 International Standard, equal to 5 International Units, and all recovered quickly (fig. 1, curve 1a). There was no doubt that B_1 was the controlling factor.

The supplements were given to each rat daily, mixed with autoclaved yeast and fed by mouth with a graduated hypodermic syringe. Groups 2, 3, and 4 (fig. 1, curves 2, 3, 4) received .25, .5, and 1 International Unit of B_1 respectively. Those on .25 unit slightly more than maintained their weights; group 3, (.5 unit), and group 4, (1 unit), produced proportionately greater gains. There was a rapid decrease in weight when the supple-

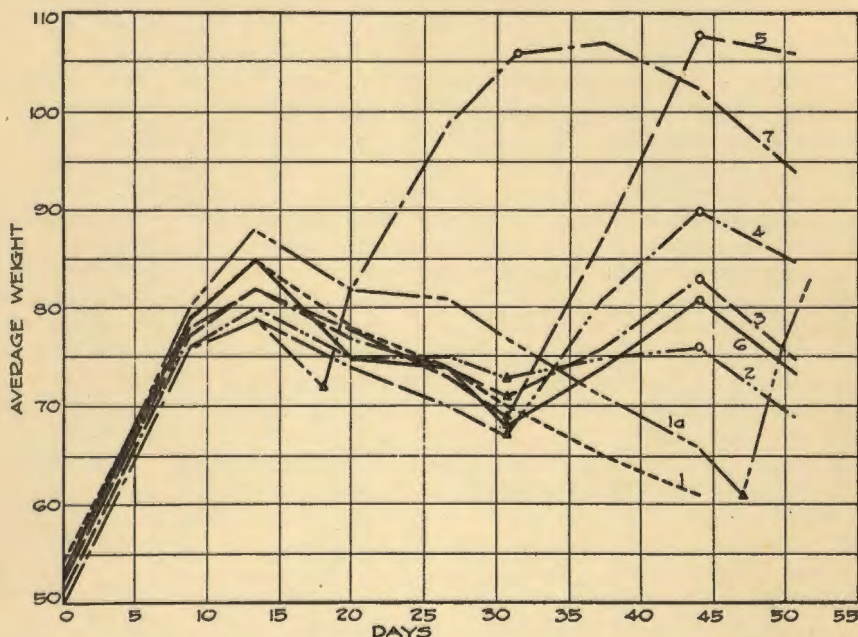


Fig. 1. Average growth curves of groups of rats fed a vitamin B_1 -free basal diet, with and without supplements.

▲ Supplement started. ○ supplement discontinued.

Curve 1. Negative control group

" 1a. Negative control—late supplement 5 International Units B_1 daily per rat

" 2. .25 International Unit B_1 daily per rat

" 3. .5 International Unit B_1 daily per rat

" 4. 1 International Unit B_1 daily per rat

" 5. 500 mg. sterile, "inorganic" Lemna daily per rat

" 6. 500 mg. non-sterile, "soil" Lemna daily per rat

" 7. 200 mg. non-autoclaved yeast daily per rat

ment was discontinued (fig. 1, curves 2, 3, and 4). Curves 5 and 6, figure 1, represent the recovery of two rats, each receiving 500 mg. per day of the Lemna grown under sterile conditions in inorganic solutions, and under non-sterile conditions in the soil-water mixture, respectively. The increase in weight and the return of muscular coordination showed the presence of B_1 in both cases, and the indication was that the larger amount of B_1 was formed in the sterile Lemna which had received no organic matter.

SERIES 2

In the second series 23 rats were used. More Lemna was available, but it had been dried and stored for almost twice as long as in the first series, and some loss of the B_1 content was evident. The results of this test confirmed the preliminary experiment. The depletion ration of

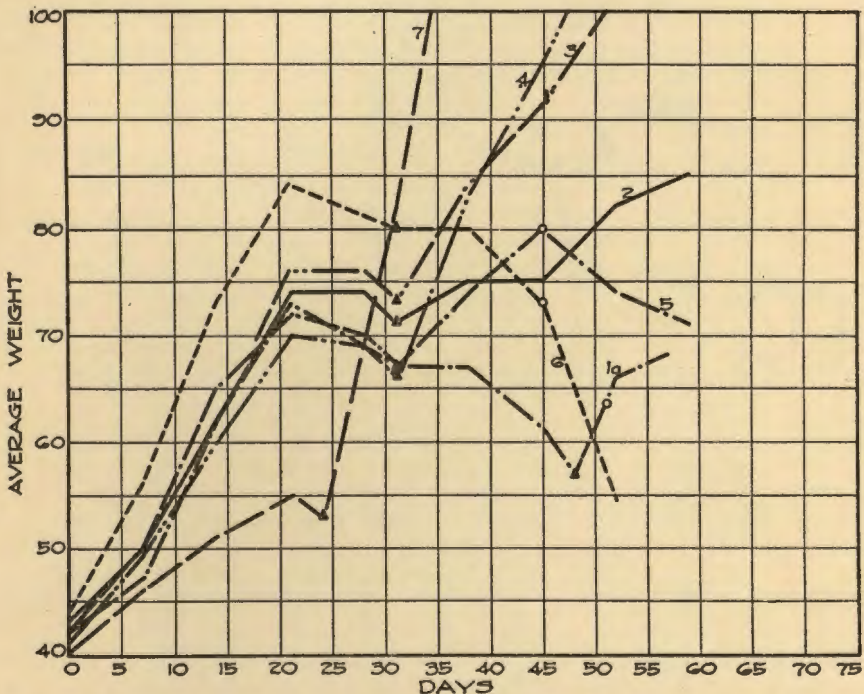


Fig. 2. Average growth curves of groups of rats fed a vitamin B_1 -free basal diet, with and without supplements.

▲ Supplement started. ○ supplement discontinued.

- Curve 1a. Negative control group—late supplement
- " 2. .5 International Unit B_1 daily per rat
- " 3. 1 International Unit B_1 daily per rat
- " 4. 2 International Units B_1 daily per rat
- " 5. 250 mg. sterile "inorganic" Lemna daily per rat
- " 6. 500 mg. non-sterile, "soil" Lemna daily per rat
- " 7. Non-autoclaved yeast

Evans, Lepkovsky and Murphy (10) was used. Each rat was given daily 500 mg. of autoclaved yeast and 50 mg. of cod liver oil, together with the supplement and a part of the basal ration. After this was consumed, free access was allowed to the basal ration.

Curve 7, figure 2, shows the positive control group and the rapid increase in weight when non-autoclaved yeast was included in the diet. The negative control group, curve 1a, at 48 days showed marked muscular incoordination, but this disappeared rapidly, and weight increased following daily supplementation with 2 International Units per rat. Groups 2, 3, and 4 received per rat .5, 1, and 2 units of the Standard Vitamin B₁ preparation, respectively. The growth curves are shown in figure 2, curves 2, 3, and 4. Group 5, consisting of 4 rats, received 250 mg. each of the dry sterile 'inorganic' Lemna; curve 5, figure 2, shows the rapid gain in average weight. The downward trend of curve 6 was arrested for a few days, following supplementation with 500 mg. of the non-sterile Lemna from the soil mixture. When the supply of this Lemna was exhausted, the rate of weight loss became greater. In this connection an interesting comparison was made with alfalfa, dried and baled in the field. Nine hundred milligrams of this was needed per day per rat before a slight increase was found in the weight of vitamin B₁-depleted rats.

The results of the two series point definitely to the fact that Lemna manufactures vitamin B₁ whether supplied with organic matter or not. In these experiments the plant produced more of the vitamin when no organic matter was supplied and when no microorganisms were present, but with the small amounts of material involved the conclusion cannot be drawn that this always would be the case. Whether the Lemna is completely autotrophic would depend upon its behavior with this and other accessory substances, and while some work has been done along this line, vitamin B₁ has not yet been tested.

VITAMIN C

Virtanen (20) grew peas in sterile culture and, from the action of vitamin C in increasing dry weight, drew the conclusion that the vitamin was a plant hormone. Van Hausen (12) found seedlings of peas, from seeds treated with vitamin C, increased their rate of growth, and Havas (13) reported similar results, but with decided variations in the effect on different plants.

In the determination of vitamin C in Lemna several difficulties were encountered in the quantitative determination. The work was done a few years ago (Frahm, 11), and no opportunity has presented itself to go further into the matter. There was no doubt, however, that the Lemna grown under sterile conditions, free of microorganisms and in the absence of all organic matter, did produce vitamin C, but whether more or less than non-sterile Lemna on soil-water mixtures was not determined.

Investigation of the vitamin C content was carried out by chemical methods. Both iodine and 2, 6-dichlorophenolindophenol were used as oxidizing agents. The plant material was extracted according to the procedure of Bessey and King (1) and Birch, Harris, and Ray (2). Both 8 per cent aqueous solutions of hot acetic acid and of trichloroacetic acid were used, and both produced colored extracts from Lemna—reddish from the 'soil' cultures and yellow from the sterile 'inorganic' cultures.

The colors were removed by the mercuric acetate treatment of Emmerie and van Eekelen (9). The reducing values averaged about four times greater for iodine than for the dye. Results, however, were irregular, although a considerable loss of the vitamin from storage was indicated.

SUMMARY

1. Lemna, grown in inorganic solutions, without organic matter, and in the absence of microorganisms, cured xerophthalmia and increased the weight of rats which had been restricted to a vitamin A-deficient diet.
2. Lemna, grown under the same conditions, produced vitamin B₁, as shown by the response of rats fed a vitamin B₁-deficient diet supplemented with the Lemna.
3. More vitamin B₁ was formed in the Lemna grown under the sterile conditions without organic matter than in the plants in a soil-water solution containing microorganisms and organic material.
4. Vitamin C was indicated in the sterile 'inorganic' Lemna by both iodine and 2, 6-dichlorophenolindophenol titrations.
5. In all three cases storage decreased the quantity of vitamin in the plants.

LITERATURE CITED

1. BESSEY, O. A., AND C. G. KING
1933. Jour. Biol. Chem. 103:687-698.
2. BIRCH, T. W., L. J. HARRIS, AND S. N. RAY
1933. Nature. 131:273-274.
3. BONNER, J.
1937. Bot. Review. 3:616-640.
4. BOTTOMLEY, W. B.
1917. Proc. Roy. Soc. London. 89B:481-507.
5. CHASE, E. F., AND H. C. SHERMAN
1931. Jour. Amer. Chem. Soc. 53:3506-3510.
6. CLARK, N. A.
1924. Ind. Eng. Chem. 16:249-250. 1925. Jour. Phys. Chem. 29:935-941.
1926. Plant Physiol. 1:273-279. 1932. Iowa State Coll. Jour. Sci. 7:13-16.
1935. Jour. Amer. Soc. Agron. 27:100-103.
7. CLARK, N. A., AND B. H. THOMAS
1934. Science. 79:571-572.
8. CLARK, N. A., AND E. M. ROLLER
1931. Soil Sci. 31:299-309.
9. EMMERIE, A., AND M. VAN EEKELLEN
1934. Biochem. Jour. 28:1153-1154.
10. EVANS, H. M., S. LEPKOVSKY, AND E. A. MURPHY
1934. Jour. Biol. Chem. 107:429-437.
11. FRAHM, E. E.
1935. Thesis, Library, Iowa State College.
12. VAN HAUSEN, S.
1935. Nature. 136:516.

13. HAVAS, L.
1935. *Nature*. 136:435.
14. HITCHCOCK, A. E., AND P. W. ZIMMERMAN
1935. *Boyce Thompson Inst.* 7:447-476
15. HUNT, C. H.
1927. *Ohio Agr. Exp. Sta. Bul.* 415.
16. KÖGL, F., AND H. ERXLEBEN
1934. *Zeit. Physiol. Chem.* 227:51-73.
17. McCARRISON, R.
1926. *Indian Jour. Med. Res.* 14:351-378.
18. MOCKERIDGE, F. A.
1924. *Ann. Bot.* 38:723-734.
19. SAEGER, A.
1925. *Jour. Gen. Physiol.* 7:517-526.
20. VIRTANEN, A. I.
1936. *Nature*. 137:779-780.
21. VISWA NATH, B.
1932. *Some aspects of plant nutrition.* Agr. Res. Inst. Coimbatore.
22. WENT, F. W.
1935. *Bot. Review.* 1:162-182.

A METHOD FOR RECORDING EVAPORATION FROM A POROUS ATMOMETER CUP¹

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A continuous record of the amount and rate of evaporation is useful in the study of various water relations for plants and soil. Evaporation from a porous atmometer cup integrates to some extent the effects of radiation intensity and air velocity, humidity, and temperature and consequently, these cups have come into common use (4). Chalkley and Livingston (1) have described a method for automatically recording the rate of evaporation from a porous cup. Their apparatus simply records the pressure drop across a flow resistance, inserted in the line between the supply water reservoir and the porous cup. Christensen, Veihmeyer and Givan (2) have shown that at constant temperature the pressure drop across such a resistance is accurately proportional to the evaporation rate, but that appreciable corrections must be applied to compensate for viscosity changes that accompany ordinary temperature fluctuations.

Morris and Durrell (5) have devised a recording atmometer that is extremely simple to construct and gives directly the cumulative curve for the evaporation from a porous cup. With their instrument the recording pen is attached to a metal float and thus records directly the level of the water in the atmometer supply reservoir. It is difficult, however, to obtain with appreciable accuracy a record of the evaporation rate from such a curve.

The authors here describe the use of a recording drop counter for measuring the flow of water to an atmometer cup. The operating principle for this flow meter which has been described elsewhere (3, 6) consists in causing water enroute from the reservoir to the atmometer to form in drops at a dropper tip located in a kerosene filled chamber. By means of direct mechanical coupling (6) the time at which each drop falls from the dropper tip is recorded on a chronograph. These drops then pass downward through the kerosene-water interface and on to the atmometer cup.

The effects of temperature and flow rate upon drop size have already been described (3). Temperature effects are relatively small, being less than one per cent decrease in drop size for a rise in temperature of five degrees Centigrade. The dependence of drop size on flow rate is more serious and requires the use of a calibration curve. Inconvenience from this source, however, may be minimized in certain graphical treatments of the data. In a greenhouse study (7) where this type of flow gauge was used to give a continuous record of atmometer-cup evaporation-rate, the

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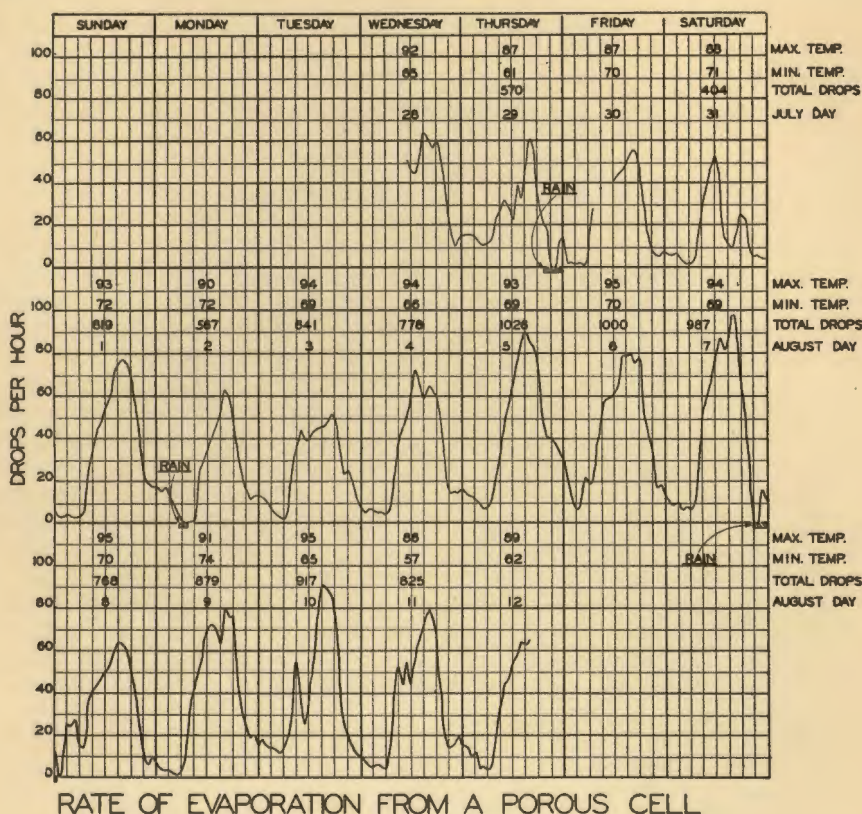


Fig. 1.

dropping rate was plotted against time on a linear scale. A non-linear scale based on the drop-size calibration-curve and giving evaporation rate in cubic centimeters per hour was then placed beside the dropping rate scale as an aid in interpreting the curve. For a flow rate recorder of this type the chronograph must be analyzed before either the flow rate or the cumulative curve can be plotted. It does have the advantage that both of these curves may be obtained with some precision.

Figure 1 shows a record of evaporation rate that was taken during the summer of 1937 at the Soil Conservation Service Experiment Station at Clarinda, Iowa. The porous cell used¹ was mounted one foot above a grass sod where it would have continuous exposure to the sun. Standard one-eighth inch copper tubing was used to make connection to the drop counter and water reservoir which were located in a field house approximately 20 feet from the cup. A drum speed of 20 inches per hour was used, making it possible to put a 48-hour record on one chart 20 inches long and 6 inches wide. The curves in the figure were obtained by plotting the number of drops during an hour against the time at the end of the hour. The curves

¹The porous cell was fired from preston clay. It was light red in color and cylindrical in shape, being 5.8 cm. in diameter and 17 cm. high.

thus show the hourly average evaporation rate. No correction was made for the effect of temperature or flow rate on drop size.

For the period of record it is seen that the minimum evaporation rate usually occurred between 3 and 6 o'clock in the morning and the maximum evaporation rate usually occurred between 3 and 6 o'clock in the afternoon. Because of the many factors affecting evaporation there is no close relation between the daily maximum air temperature and maximum evaporation rate or total evaporation for the day. The graph shows that usually there occurred intermediate minima in the evaporation rate some time between sundown and midnight.

Rainfall periods as determined from recording rain gage records are shown as rectangular cross-hatched areas under the time axes in the figure. No provision was made to prevent rain water from entering the porous cell. It is interesting to note that the drop counter recorded very appreciable amounts of evaporation during periods when the recording rain gage charts indicated continuous rain. The authors know that the sharp rise in the evaporation rate which occurred during the more or less continuous four-hour rain of August 7 was occasioned by the abrupt beginning of a hard wind, because at that time they got out to repitch the tent in which they had been sleeping.

The authors plan to obtain continuous records of water loss from plants by feeding nutrient solution through a drop counter to plants growing in liquid culture in a sealed root container.

REFERENCES

- (1) CHALKLEY, H. W., and B. E. LIVINGSTON
1929. Atmometer rates read instantaneously and an automatic continuous recorder for rate fluctuation. *Ecology*, 10:37-46.
- (2) CHRISTENSEN, J. E., F. J. VEIHMEYER, and C. V. GIVAN
1930. Effect of variations in temperature on the operation of the instantaneous reading atmometer. *Ecology*, 11:161-169.
- (3) IVIE, J. O., and L. A. RICHARDS
1937. A meter for recording slow liquid flow. *Rev. of Sci. Instr.*, 8:86-89.
- (4) LIVINGSTON, B. E.
1935. Atmometers of porous porcelain and paper and their use in physiological ecology. *Ecology*, 16:458-472.
- (5) MORRIS, M. S., and L. W. DURRELL
1932. A recording atmometer. *Ecology*, 13:100.
- (6) RICHARDS, L. A., M. B. RUSSELL, and O. R. NEAL
1937. Further developments on apparatus for field moisture studies. *Soil Sci. Soc. Am. Proc.*, 2:55-63.
- (7) RICHARDS, L. A., and M. B. RUSSELL
1937. Apparatus for studying water relations in potted plants. *Trans. Am. Geophysical Union*, 18th Annual Meeting, 588-592.

THE HIGH FREQUENCY GLOW DISCHARGE IN HYDROGEN EXCITED THROUGH INTERNAL ELECTRODES

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Although the alternating current discharge has been known for almost as long as the D. C. type, it is so complex that no theory has yet appeared which satisfactorily explains all the observed phenomena. For this reason, investigations of high frequency discharge characteristics have been limited usually to the experimental determination of sparking and maintenance potentials.

Two types of discharge are known as high frequency. In one, the gas is excited by the electro-magnetic field surrounding a coil carrying a high frequency current. In the other, the gas is excited by applying to it the high frequency voltage appearing across a source such as the plate coil of a vacuum tube oscillator. In this type, known as the electro-static discharge, the gas, enclosed in a glass container, may have the exciting voltage applied through external sleeve electrodes as was done by Fox and Bachman (1) or, as in this investigation, the energy may be fed directly to the gas through electrodes sealed inside the container. In the latter case, energy losses in the glass and consequent distortion of the electric field in the vicinity of the electrodes can be materially diminished.

THEORY

A theory of the glow discharge excited electrostatically has been proposed by J. Thomson (2). On the basis of a sinusoidal electric field (E), the displacement of a free electron is, according to this theory:

$$X = \frac{Ee}{2\pi v^2 m} \left(\frac{1}{2\pi} [\cos \phi - \cos(2\pi vt - \phi)] + t \sin \phi \right) \quad (1)$$

in which ϕ is a phase angle, v is the frequency of electric field variation, and e/m is the ratio of the charge to the mass of the electron. This equation results from the reasonable assumption that if the typical free electron begins to move when the electric field strength is $E \cos(-\phi)$, the velocity necessary for ionization will be most quickly attained if it is acquired in a time t_1 , such that the field at this time is $E \cos(+\phi)$, for then,

$$\int_0^{t_1} \cos(2\pi vt - \phi) dt$$

is a maximum. From equation (1), the maximum displacement of the electron is:

$$X = \frac{Ee}{2\pi v^2 m} \quad (2)$$

For a discharge tube with plane parallel electrodes, the equation for the sparking potential is of the form:

$$V_s = A + bpd \quad (3)$$

where A is a function of the electrodes and b is a function of the gas, p is the pressure, and d is the interelectrode distance. If V equals the potential through which a positive ion must fall to produce a certain number of electrons at the cathode surface, and V_0 be a quantity proportional to the ionization potential of the gas, the electron fall in potential V_e takes place in a distance

$$\frac{L}{4\sqrt{2}} = \frac{K}{4\sqrt{2}p},$$

and Thomson's theory shows

$$V_s = V_e + \frac{V_0}{K} p \left(d - \frac{K}{4\sqrt{2}p} \right)$$

while further analysis allows the deduction that:

$$A = V_e - \frac{V_0}{4\sqrt{2}}, \quad b = \frac{V_0}{K}$$

The analysis implies that a space charge is built up which permits the positive ions to release a sufficient number of electrons from the cathode to start the current. It also implies an abnormal fall in potential in the neighborhood of the cathode brought about by this space charge, but a uniform potential gradient throughout the remainder of the interelectrode distance d . Such implications appear reasonable in view of the difference in mobilities of the electrons and positive ions.

For moderately high frequencies of excitation, where the amplitude of oscillation is quite large, Thomson obtains for b the experimental value 18 which suggests the electron travels twelve times the kinetic theory mean free path before ionizing. This means that a smaller number of electrons can excite a discharge in the AC than in the DC type of discharge; but it also implies that, when the electronic amplitude of oscillation falls

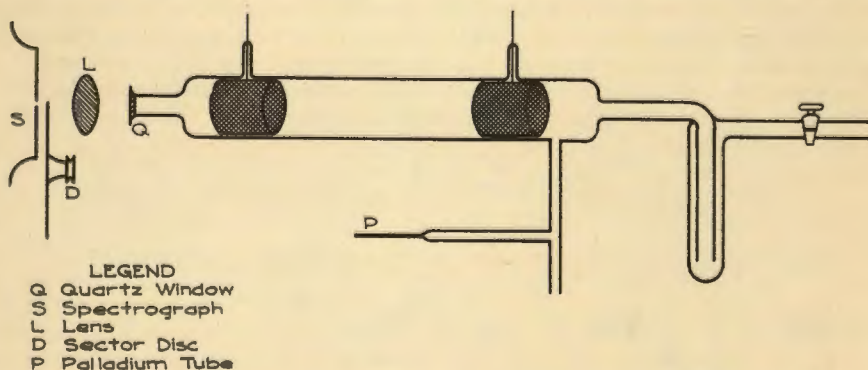


Fig. 1. Experimental arrangement of apparatus for photographing the hydrogen spectrum.

to the order of $12L$ (L is the mean free path of an electron), the value of the field strength necessary to produce a discharge must increase.

Although strictly speaking this theory was developed for striking potentials, it seems probable that its general features can be applied as well to maintenance potentials. This has been done in the present investigation, in which the intensity variations of certain lines in the Balmer series of hydrogen, excited by high frequency voltages applied through internal electrodes, have been studied as functions of pressure and frequency.

EXPERIMENTAL PROCEDURE

The gas system (Fig. 1) was entirely of Pyrex except for a fused quartz window, Q , connected to one end of the discharge tube by means of a graded seal, and a palladium tube, P , similarly connected to a side tube. An oil diffusion pump of the vertical stream type, charged with apiezon oil B, and a hyvac fore pump made up the evacuating system. The discharge tube, 50 cm. long and 5 cm. in diameter, was isolated from the pumping system by a liquid air trap. Especially prepared graphite electrodes, in the form of cylindrical shells 4 cm. in diameter and 4 cm. in length, were mounted in the tube 30 cm. apart. Hydrogen was admitted to the system by diffusion through palladium, the pressure being read by a Pirani gauge.

The high frequency voltage was generated by a push pull oscillator powered with type 852 tubes and was measured directly by a cathode ray oscillograph. The load on the oscillator, imposed by the oscillograph, was negligible and did not affect its efficient operation. Oscillation frequencies were measured by General Radio wave meters, types 22L and 358.

Spectrograms of the discharge were made with a Bausch and Lomb medium quartz spectrograph in conjunction with a logarithmic sector disk similar to that described by Twyman and Simeon (3). The disk was constructed to the equation $-\log \theta = 0.2l$ and differed from theirs in having two symmetrically placed spirals instead of one. The value of θ represents the circumferential aperture at a distance l measured inward radially from the outermost part of the disk. The disk was driven at 3,000 r.p.m. The spectrum line lengths, photographed on Wratten and Wainwright panchromatic plates, were measured consistently to 0.1 mm. All plates were from the same emulsion batch and were developed in fresh stock solution under constant temperature and time conditions. Line intensities were reduced to unit exposure and the line lengths computed according to the equation $\log t_1 - \log t_2 = 0.2(l_2 - l_1)$, which means that, for constant exposure, the length of a spectrum line will vary as the intensity of that line. Spectroscopic data were obtained for various frequencies of excitation up to 5×10^7 cycles per second and for pressures from 5 to 300 microns. A peak potential of 1,500 volts was maintained between the electrodes on all runs.

RESULTS AND DISCUSSION

The results, in graphical form, (figure 2), show how the intensities of the first six lines of the Balmer series of hydrogen vary with pressure and frequency of excitation. It may be said, in general, that for each line there exists an optimum pressure and one or more favorable frequencies of excitation. The agreement between these curves and those of Fox and

and Bachman is apparent. The decrease in conductivity for wave lengths of excitation above 25 meters and below 6 meters has been recorded by Brasefield (4) and Thomson (2).

The intensity of the shorter wave length lines practically reaches that of H_α for low pressure. This suggests that the population ratios for the energy states involving these lines is closely connected with the pressure. Lowering the pressure results in longer mean free paths so that the particles attain higher velocities between collisions. Thus, their heat motions become less important compared to their field directed speeds so that the probability of excitation of the higher terms in the series approaches that of H_α .

For stable high frequency operation, sufficient positive ions must be produced by collision of electrons with gas molecules within a few mean free paths and within a time not greater than half a cycle to fulfill the con-

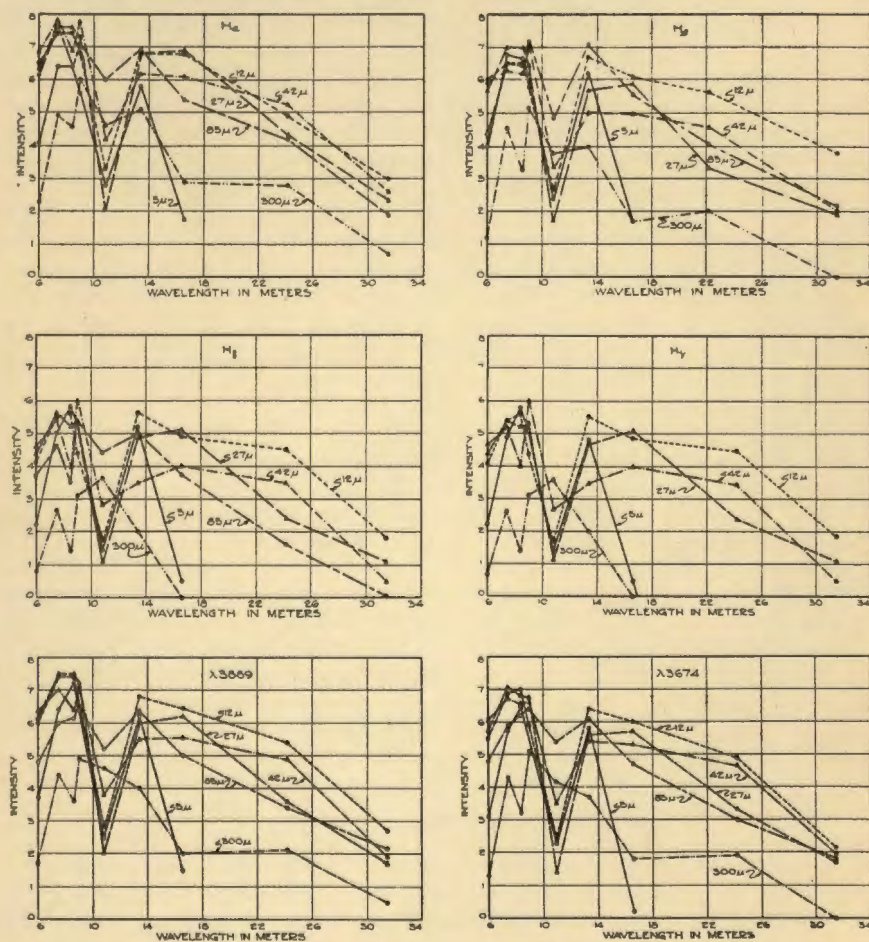


Fig. 2. Intensity vs wave length of excitation curves for different pressures for first six lines of Balmer series.

ditions for a self-maintained discharge. Obviously, at collision, the electron must have sufficient energy to ionize. When the wave length of excitation is increased, the time required for the electric force to reach its maximum value increases and hence a longer time is required for the electron to reach ionizing velocity. Thus, the efficiency of the discharge should increase with the frequency of excitation except at frequencies for which the electron oscillation amplitude falls to the order of the electron mean free path. Here the discharge efficiency would be expected to decrease.

If Thomson's Theory is applied to the present investigation, three assumptions are necessary:

1. That the electrodes are plane parallel.
2. That the cathode sheath potential gradient is of the order of 325 volts per centimeter.
3. That conditions for minimum sparking potential are the same as for discharge maintenance with maximum efficiency.

Because of the large interelectrode distance, the error introduced by the first assumption is negligible. The second is in accord with numerous probe studies made by Langmuir in direct current discharges, while Brasefield (4) and others have shown that a discharge has its maximum conductivity under the same conditions that give minimum striking potential.

A simple calculation indicates that, at the optimum pressure (30 microns), the kinetic theory mean free path is that of the order of 2.58 cm., so that, according to the discharge theory outlined, the electronic mean free path is 31 cm. (12L). If these values are substituted in equation (2), the optimum frequency of excitation is 5.5×10^7 cycles per second (wave length 5.4 meters). The experimental curves suggest a minimum in this region. Resonance phenomena of some sort probably explain the minimum at 10.8 meters shown in the curves.

The relation of the interelectrode distance and electron amplitudes of oscillation for excitation are: 2d, 8d, and 16d for the maxima; and 1d, 4.5d, and 9d for the minima (5).

The electronic mean free path for the optimum pressure is of the same order of magnitude as the electrode separation for:

$$p = \frac{12K}{d} = \frac{12 \times 7.74 \times 10^{-2}}{30} = 33 \text{ microns.}$$

These results indicate a close relation between the pressure, the tube dimensions, and the excitation frequency for maintenance of a high frequency discharge. Any variation from optimum conditions tends to increase or decrease the intensity of the spectrum line depending on whether it was originally a maximum or minimum. If the dimensions of the tube were changed, it would be expected the optimum maintenance conditions would change also, which indicates that Townsend's law of similitude is valid for this type of discharge.

REFERENCES

1. Fox, G. W., and C. H. BACHMAN
1936. Line intensity variations in the hydrogen high frequency glow discharge. *Phys. Rev.* 50: 939-942.

2. THOMSON, JOHN
1937. Sparking potentials at ultra high frequencies. *Phil Mag. Series 7.* 23:1-24.
3. TWYMAN, F., and F. SIMEON
1929. The logarithmic sector wedge and its use in quantitative spectrum analysis. *Trans. Optical Soc. London*, 31:169-181.
4. BRASEFIELD, C. J.
1930. The conductivity of a high frequency discharge in hydrogen. *Phys. Rev.* 35:1073-1079.
5. BURDINE, N. T.
1937. High frequency glow discharge with internal electrodes. Unpublished Thesis, Library, Iowa State College, p. 34.

LOWER COST DIETS FOR HATCHERY TROUT

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From the Iowa State Conservation Commission

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One of the major projects in the fish management plan of the Iowa State Conservation Commission is the production and stocking of trout at least seven inches long, the minimum legal length. In fact, the trout stocked in Iowa streams range from eight to ten inches in length. With such a trout program feeding costs are ever before the hatchery manager.

Prices of beef heart, a favored food item, have ranged from \$.08 to \$.125 per pound in the past few years. Certain commercial trout foods have been as high as \$.065 per pound. These prices indicate that in the production of trout for a reasonable stocking program, foods of lower prices must be sought.

Many trout feeding experiments have been made by skilled fisheries workers to determine the kind of food which is most readily taken and which would produce best growth results economically under optimum conditions.

The writer (unpublished manuscript) obtained with starved fingerling yellow pike-perch (*Stizostedion vitreum*) results that were not comparable with those noted by Titcomb and associates (1928) on starved trout which readily recovered from periods of starvation. No doubt anatomical differences in the type of stomachs of different fish play an important role not only in type of foods elected but in ability to recover from periods of starvation.

McCay and associates (1931) indicated in studies covering nutritional requirements of trout that certain diets must be balanced and that protein content of feed must be kept within certain limits.

McCay and Tunison (1934) worked out growth rate curves with brook trout (*Salvelinus fontinalis*) which showed that varying percentages of proteins and fats in several diets definitely affected the growth rate of trout.

Fielder and Samson (1935) stated that bones of canned carp might be beneficial as food particles; but this was not verified by the following described experiment.

In an attempt to find less expensive food for trout that would give satisfactory growth results, two experiments in feeding trout were inaugurated by the writer in 1936. In the first experiment, designated by "A", 3,000 brook trout were fed. In the second experiment, designated by "B", 450 brown trout (*Salmo fario*) were used. As the Iowa State Conservation Commission maintains a trout hatchery at the Backbone State Park near Strawberry Point, the feeding work was carried on there. The feeding, weighing and recording of data was personally handled by R. B. Cooper, Fish Culturist, in charge of the Backbone Station.

A comparative study was made of the values of (1) beef heart, (2) beef hearts, 50 per cent, and ground cooked carp and buffalo, 50 per cent, and (3) ground cooked carp and buffalo as food for the trout. The beef

hearts were trimmed and ground fresh. The carp and buffalo were ground entire, cooked and packed in seven-pound cans. Buffalo too small to have a market value made up 80 per cent of the ground fish and the other 20 per cent was carp of four to five pounds in individual weight.

As beef hearts composed the major portion of the food used regularly at the Backbone Station, one group of fish was fed that diet for purposes of checking the results of the other two diets.

By using the mixed diet of ground fish and beef hearts it was expected that data might be secured on a cheaper food than beef hearts alone and might illustrate a fair diet with little or no serious check on growth increments. Furthermore, the diet of ground cooked fish alone might demonstrate an inexpensive food for a given period of time and at the same instance furnish an outlet for rough fish disposal.

By using two different ages and sizes of trout it was thought data might be obtained on the practicability of mixed foods at different growth periods.

The food was weighed daily and equal volumes by weight were placed in each feeding trough. The amounts of feed were normal feeding

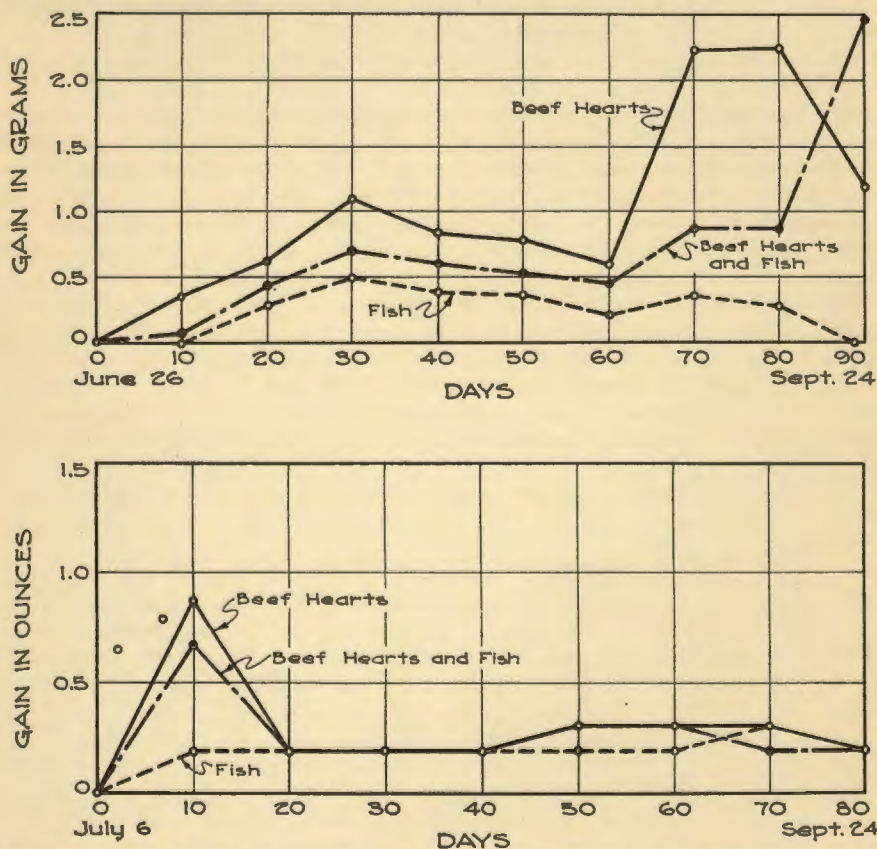


Fig. 1, A, Gain in weight of young brook trout fed on various diets; B, gain in weight of brown trout fed on various diets.

portions, and the increases in food weight were made on the fish-weighing dates.

In Experiment "A" the fish were weighed at regular ten-day intervals. Three troughs were used with 1,000 brook trout in each trough. These fish were uniform in size, about six months old, and all in a normal and healthy condition when the experiment was started. The water temperature, 50 degrees Fahrenheit, remained constant throughout the experimental period. Variables such as acidity, temperature, and oxygen content of the water were eliminated easily as a deep natural spring furnished the water supply, which was piped from the emerging point directly to the hatchery troughs. Because the temperature remained so uniformly constant, it was assumed that no marked change occurred in the oxygen content. The pH of this water supply ranged from 7.3 to 7.4 over a yearly period.

The Iowa State Department of Agriculture made a commercial feed analysis of the canned fish which showed this product to have a protein content of 17.51 per cent, fat 11.20 per cent, ash 5.20 per cent, moisture 65.00 per cent, nitrogen-free extract 1.09 per cent, and fiber none. The cost of the canned fish was \$.035 per pound in seven-pound (No. 10 size) cans.

Experiment "A" was started June 6, 1936, and was discontinued September 26, 1938. The data are given in tables 1, 2 and 3. The fish weight in grams was obtained in the following manner. At intervals of 10 days five or six random groups of 100 trout were taken from each trough and weighed separately. The average of these weights for 100 fish was multiplied by 10 for each diet group, and thus the fish weight in grams in each case was computed for 1,000 trout, although some fish were lost during the 90-day period. The weight gain in grams was found by subtracting the fish weight in grams of a given weighing day from that obtained on the next date of weighing. Gain per fish in grams came from dividing the weight gain in grams for a given 10-day interval by 1,000. From the beef heart diet group 57 trout were lost, from the heart and fish diet 160, and from the cooked fish diet 464.

Attention is called to figure 1, A of Experiment "A". On all three diets, for the first 30 days the growth rate shows an almost steady daily

TABLE 1. Experiment "A". Diet of beef hearts

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,860	360	.36
7-16	10	60	2,540	680	.68
7-26	10	80	3,660	1,120	1.12
8-5	10	80	4,480	820	.82
8-15	10	80	5,380	900	.90
8-25	10	122	6,000	620	.62
9-4	10	140	8,250	2,250	2.25
9-14	10	140	10,500	2,250	2.25
9-24	10	140	11,700	1,200	1.20

TABLE 2. *Experiment "A". Diet of beef hearts and cooked fish*

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,580	80	.08
7-16	10	60	2,020	440	.44
7-26	10	80	2,720	700	.70
8-5	10	80	3,330	610	.61
8-15	10	80	3,870	540	.54
8-25	10	122	4,310	440	.44
9-4	10	140	5,190	880	.88
9-14	10	140	6,060	870	.87
9-24	10	140	8,460	2,400	2.40

increase except on the canned fish diet. After that period another 30-day interval shows a definite decrease in gain, and then again a sharp 10-day increase in gain, which levelled off for another 10-day period and then made a sharp decrease in the two diets of beef and fish alone, but showed a remarkable increase in the combined beef and fish diet in the last 10 days. Just why there was such a variation in gain shown by the combination diet in comparison with the diets of beef alone and fish alone during these last 10 days of the experiment can hardly be conjectured since the diets paralleled each other in results up to this period. An explanation might be advanced that at this period the mixed diet was most suitable for nine-months-old fish and points to a cyclic diet demand. The marked fluctuations in growth in each individual trough parallel and definitely point out relative merits of the respective diets at the given ages. The results demonstrate that further study needs to be made over a longer period of time, at least 18 months, the normal age of trout at stocking time. Such a study would give better information on both determinate and indeterminate growth rates.

To the culturist feeding the fish, no sudden changes in fish failing to feed or exhibiting unusual hunger were apparent at any time on the

TABLE 3. *Experiment "A". Diet of cooked fish*

Date	Interval in days	Food weight in ounces	Fish weight in grams	Weight gain in grams	Gain per fish in grams
6-26	0	0	1,500	0	.00
7-6	10	44	1,540	40	.04
7-16	10	60	1,830	290	.29
7-26	10	80	2,350	520	.52
8-5	10	80	2,730	380	.38
8-15	10	80	3,090	360	.36
8-25	10	140	3,300	210	.21
9-4	10	140	3,640	340	.34
9-14	10	140	3,960	320	.32
9-24	10	140	3,890	-70	-.07

TABLE 4. *Experiment "B". Diet of beef hearts*

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	405	135	.9
7-26	10	120	150	435	30	.2
8-5	10	120	150	465	30	.2
8-15	10	120	150	495	30	.2
8-25	10	120	150	540	45	.3
9-4	10	120	150	585	45	.3
9-14	10	120	150	630	45	.3
9-24	10	120	150	660	30	.2

beef heart diet. The fish fed on beef hearts were in excellent condition throughout the experiment. The trout in the beef heart-cooked fish trough were not as deeply colored or quite as uniform in size at the end of the feeding experiment as those fed on beef hearts alone. The fish fed on the cooked fish alone became very uneven in size and in poor physical condition as the work continued.

The early unevenness in size became more marked as time passed and mortality increased with regularity. It was apparent that the bone particles, although soft enough for food, were unpalatable. It also appeared that young trout after striking food particles several times desisted, perhaps through fatigue, and therefore failed to continue feeding until nutritional requirements were appeased. Mortality among the fish on the cooked fish diet became so heavy in 90 days that Experiment "A" was stopped.

In Experiment "B", larger fish were used in out-door raceways. In this experiment three troughs were set up with 150 brown trout in each retainer. The following tables give feeding details and results.

In making an analysis of this experiment, figure 1, B indicates a very comparable gain after the first 20 days, and bears out other feeding experiments being conducted that better results are obtained with cooked

TABLE 5. *Experiment "B". Diet of beef and cooked fish*

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	375	105	.7
7-26	10	120	150	405	30	.2
8-5	10	120	150	435	30	.2
8-15	10	120	150	465	30	.2
8-25	10	120	150	510	45	.3
9-4	10	120	150	555	45	.3
9-14	10	120	150	585	30	.2
9-25	10	120	150	615	30	.2

TABLE 6. Experiment "B". Diet of cooked fish

Date of Weighing	Interval in days	Food in ounces	No. of fish	Food weight in ounces	Gain in ounces	Gain per fish in ounces
7-6	0	0	150	270	0	.0
7-16	10	120	150	300	30	.2
7-26	10	120	150	300	30	.2
8-5	10	120	150	330	30	.2
8-15	10	120	150	360	30	.2
8-25	10	120	150	390	30	.2
9-4	10	120	150	420	30	.2
9-14	10	120	150	465	45	.3
9-24	10	120	150	495	30	.2

fish if fed to the larger sized fish. The sharp gain the first ten days is no doubt due to the fact that these fish had less competition for food after being removed from the holding pond.

It is believed that the percentage of non-nutritious food particles, such as pieces of bone and scales, of the cooked fish, when fed to very young fish is one factor that might create an uneven growth in the early stages of the feeding experiment and might be a condition of inanition.

In making conclusions on comparative value and cost of the different foods the analysis of Experiment "B" is shown in table 7.

SUMMARY

On a cost basis, ground cooked carp and buffalo was the most economical of the three diets. However, the quality and gross pounds of fish produced is an important factor, which makes a decision necessary as to which is most desired—quality or quantity—for money expended.

The experiment demonstrated that fingerling fish can be fed on a mixed diet of beef hearts and canned fish at a saving on food cost of 28 per cent and on canned fish alone at a saving of 40 per cent without serious impairment of quality. In Experiment "A" this was negatively shown with smaller fish.

Growth rate studies point to physiological phases affecting fish cultural results as seen in ability of trout fed on canned fish to recover from prolonged feeding on a diet that is apparently lacking in some important nutritional factor.

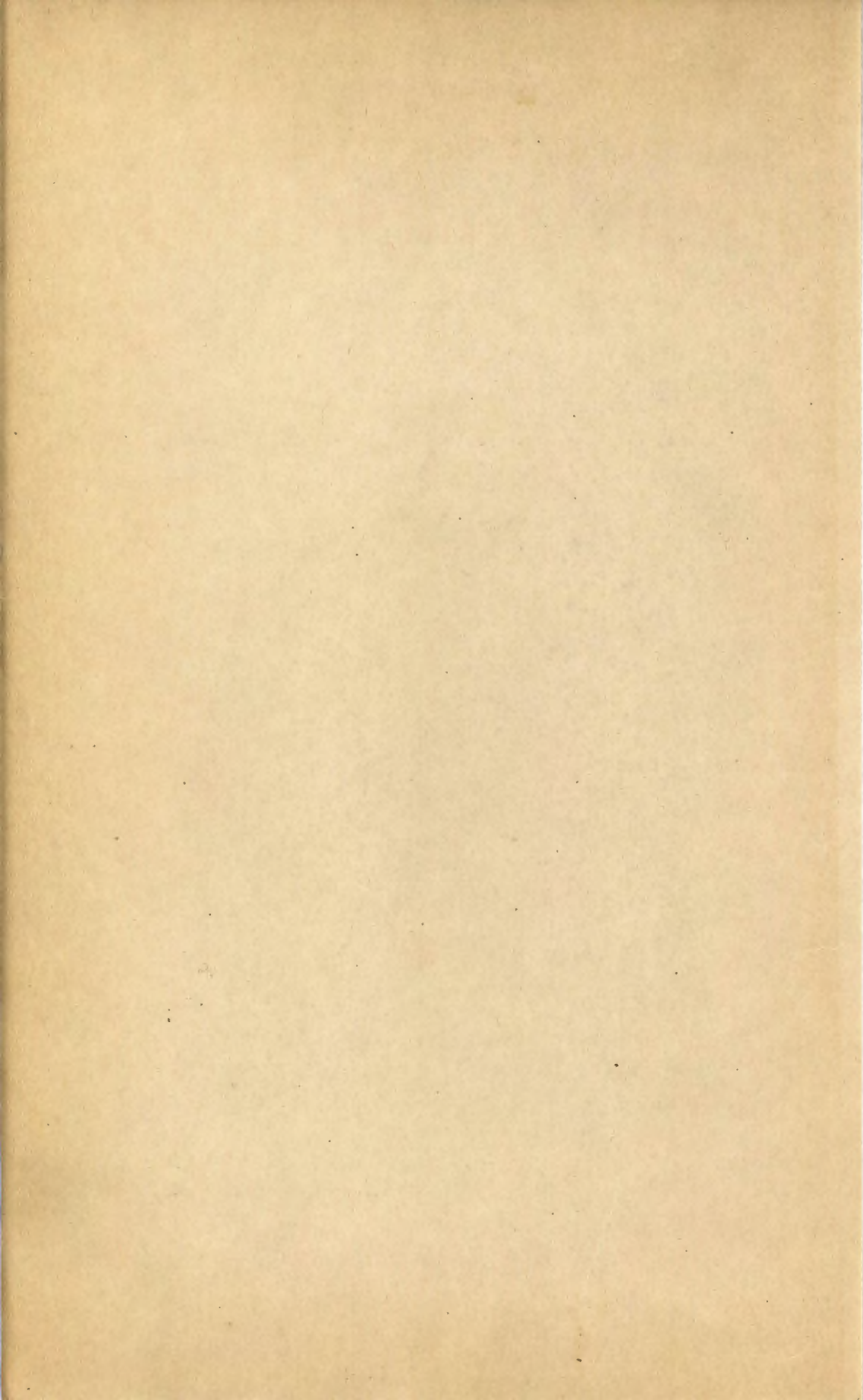
TABLE 7. Analysis of Experiment "B"

Diets	Pounds	Cost per Pound	Fish Pounds	Cost per pound of trout produced
Beef Hearts	60	\$.10	24.375	\$.25
Beef hearts and cooked fish	60	.0675	21.5625	.18
Cooked fish	60	.035	14.0625	.15

Both experiments show relative values of the different diets on growth rate and weight gain for a definite period.

LITERATURE CITED

- FIEDLER, R. H., and V. J. SAMSON
1935. Survey of fish hatchery foods and feeding practices. Memorandum No. 2526, Bureau of Fisheries, U. S. Department of Commerce, Washington, D. C.
- McCAY, C. M., A. TUNISON, MARY CROWELL, D. K. TRESSLER, S. P. MACDONALD, JOHN W. TITCOMB, and EBEN W. COBB
1931. The nutritional requirements of trout and chemical composition of the entire trout body. Transactions of the American Fisheries Society, 61:58-79.
- McCAY, C. M., and A. V. TUNISON
1934. Report of the experimental work of the Cortland Hatchery, New York State Conservation Department.
- TITCOMB, J. W., EBEN W. COBB, MARY F. CROWELL, and C. M. McCAY
1928. The nutritional requirements and growth rates of brook trout. Transactions of American Fisheries Society, 58:205-226.



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THE ELECTROMETRIC DETERMINATION OF THE SOLUBILITY OF SOME HYDROXIDES¹

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Received June 25, 1938

The present problem developed in an attempt to learn whether magnesium forms complexes with ammonium salts in dilute solutions.

Time of flow against concentration curves were made for solutions containing a fixed amount of magnesium chloride and varying amounts of ammonium chloride. These curves were found to resemble the same curve for solutions containing potassium chloride alone in varying concentrations, except that the minimum was reached at a lower concentration of the ammonium salt. This shifting of the minimum may be explained by the fact that the chloride ion, from the magnesium chloride, represses the ionization of the ammonium chloride. As no maximum was noted, through the ratio of one mole of magnesium chloride to two moles of ammonium chloride, it was concluded that no complex was formed.

The curve obtained by plotting refractometer readings for magnesium chloride-ammonium chloride mixtures, similar to those above, against concentrations of ammonium chloride gave a straight line. No conclusions, however, could be drawn from these results as mercuric chloride and ammonium chloride mixtures also gave a straight line and these mixtures were reported by Shibata, (5) from his work on absorption spectra, as containing a complex.

The work of Shibata, (5) on the absorption spectra of $\text{HgCl}_2\text{-NH}_4\text{Cl}$ mixtures was duplicated, in a somewhat abbreviated manner, in this laboratory. Using an iron spark, observations were made upon the absorption spectra of 0.1 M HgCl_2 , of 0.1 M NH_4Cl , and of a mixture of the two solutions in equal proportions. These observations were made in the region between 2300 and 3000 Å. The shifting of the absorption ends toward the longer wave lengths was very striking. The end of the absorption band of the mixture extended much more into this region than that of either of the solutions alone.

Observations were made, in the same region, upon one molar magnesium chloride, upon one molar ammonium chloride, and upon a mixture of the two solutions in equal proportions. No difference in the absorption spectra was noted. The absorption ends, however, had been shifted entirely out of this region by the hydrochloric acid which had been added to prevent the hydrolysis of the magnesium salt. Inavailability of the proper apparatus prevented this study being continued in the shorter wave length regions.

As the preceeding work gave results from which no well established conclusion could be reached electrometric titrations of MgCl_2 , by means of NaOH and by means of NH_4OH , were resorted to. The scheme used by Britton (1) of projecting the almost straight parts of the curve as straight lines and then taking the intersection of these two projections

¹ Original thesis submitted August, 1937. Doctoral thesis number 446.

as the point at which precipitation began, was used. The concentration of the hydroxyl ion was calculated from the potential and the concentration of the magnesium ion was calculated from the equivalents of base added to start the precipitation. The solubility products calculated from these data, agreed in order of magnitude with each other and with the generally accepted value. The results from the titration of 0.025 M MgCl_2 with 0.1 N NaOH gave a solubility product of 2.19×10^{-11} . Seven titrations of 0.05 M MgCl_2 with 0.25 N NH_4OH gave solubility products varying from 1.7×10^{-11} to 4×10^{-11} . These results justify the conclusion, that no complexes are formed by magnesium and ammonium salts in the dilute solutions studied.

An attempt was made to determine the solubility product of magnesium hydroxide by the electrometric titration of magnesium hydroxide with ammonium chloride, by noting the potential and the total volume at which the precipitate completely dissolved. Due to the difficulty encountered in accurately determining this point of complete solution, the results obtained by this method were not so satisfactory. The results from six determinations varied from 1.1×10^{-11} to 9.2×10^{-11} .

A further attempt to determine the solubility product of magnesium hydroxide was made, by determining the pH of a saturated solution of the hydroxide. The solubility product varied more widely in this method, values from 2.45×10^{-12} to 8.3×10^{-11} being obtained. The pH of the solution varied with the method of preparation of the hydroxide. Part of this variance was due, no doubt, to the precipitation of basic salts rather than the pure hydroxide. Solutions prepared by saturating water with magnesium oxide consistently gave results higher than those prepared from the precipitated hydroxide. These higher values check somewhat closer the generally accepted value of 3.4×10^{-11} .

Although this method gave less consistent results than either of the other methods, it was thought to be worth while to investigate it using other hydroxides as the procedure was much simpler. Using this method, values of 1.115, 1.147 and 1.141 in grams per liter were obtained for solubility of calcium oxide at 25°C. These values compare favorably with the value of 1.148 grams per liter of water calculated from Leyson and Moody's (3) data at the same temperature. The solubility, at 25°C, of thorium hydroxide prepared by saturating a solution with thorium dioxide was found to be 2.4×10^{-5} g. per liter. This value checks in order of magnitude with that of 2×10^{-5} reported by Spitzin (7) for the same temperature. Lanthanum calculated as the sesquioxide gave 0.008 g. at 25°C per liter as compared to 0.004 g. at 29°C reported in the Handbook of Chemistry and Physics (2). The original source of this data on Lanthanum could not be found. The solubility product of zinc hydroxide determined in this manner was found to be 1.69×10^{-21} which agrees with Britton's (1) value of 10^{-21} but differs considerably from the generally accepted value of 1.8×10^{-14} . The solubility of strontium oxide was found to be 0.561 g. to 0.644 g. per 100 ml by this method. The pH of the solutions giving these values varied by 0.06. From the results given above it can be seen that this method is useful only in determining the order of solubility as a small difference in pH makes a large difference in the calculated value for the solubility.

The solubility of strontium oxide as determined varied so much from Sidersky's (6) value of 0.82 g. per 100 grams at 25°C that it was

decided to check the solubility by ordinary analytical methods. The solubility was determined by the ordinary volumetric method, by precipitation as strontium sulphate and by an evaporation method in which the strontium hydroxide solution was treated with an excess of six normal sulphuric acid, and then evaporated to dryness and ignited over a Bunsen flame. Some difficulty was encountered in obtaining checks closer than two or three parts per thousand, by the three methods, on individual samples. The mean values, however, from a number of determinations checked satisfactorily. Eighteen determinations by the evaporation method gave a mean of 0.8547, twenty-eight determinations by precipitation gave a mean of 0.8552 and fifteen volumetric determinations gave a mean of 0.8544. An approximate mean from the three methods is 0.855 g. per 100 ml or 0.850 g. per 100 grams. This value is much closer to Riedel's (4) value of 0.849 g. per 100 ml than to Sidersky's (6).

SUMMARY

1. The viscosity data, although their interpretation is somewhat questionable, offer fair evidence that no complex is formed by magnesium and ammonium salts in dilute solution.

2. The refractive indices of solutions were found to be unsuitable as evidence of the nonformation of complexes.

3. The absorption spectra although somewhat limited in scope gave no evidence of complex formation between magnesium and ammonium salts. This study is worthy of further work.

4. Agreement between the electrometric titration of magnesium chloride using sodium hydroxide and the same titration using ammonium hydroxide offers strong evidence that no complex is formed in the latter case.

5. The electrometric titration of magnesium hydroxide using ammonium chloride is not very satisfactory due to difficulty in observing accurately the disappearance of the precipitate.

6. The determination of the solubility of magnesium oxide, thorium dioxide, lanthanum hydroxide, calcium oxide and strontium oxide through the determination of the pH's of the saturated solution was found to give results agreeing in order of magnitude with the generally accepted values. The results on zinc oxide were somewhat questionable.

7. The solubility of strontium oxide at 25°C is established as 0.855 g. per 100 ml.

LITERATURE CITED

1. BRITTON, H. T. S.
1925. *J. Chem. Soc.* 127:2110-2159; 1932. *Hydrogen Ions.* pp. 295-231. Chapman and Hall, Ltd., London.
2. HODGMAN, C. D.
1935. *Handbook of Chemistry and Physics.* p. 372. Chemical Rubber Publishing Co., Cleveland.
3. LEYSON, L. T., AND G. T. MOODY
1903. *J. Chem. Soc.* 93:1767-1775.
4. RIEDEL R.
1906. *Z. physik. Chem.* 56:250.

5. SHIBATA, Y.
1922. Japan J. Chem. 1, 1-18.
6. SIDERSKY, D.
1921. Bull. assoc. chim. sucr. dist. 39, 167-177.
7. SPITZIN, V. I.
1917. J. Russ. Chem. Soc. 49, 357-370. Original not seen. Abstracted in C. A. 17, 3291 (1923).

METALATION OF CYCLIC COMPOUNDS¹

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Metalation has been defined as the reaction wherein a hydrogen attached to a carbon atom is replaced by a metal to form a true organo-metallic compound². The agent used to bring about this reaction might be a free metal, an inorganic compound, or an organometallic compound. A survey of representative examples of metalation was presented, with particular emphasis upon the course of the reaction and the conditions under which it took place. The investigation of examples of metalation was undertaken in order to correlate details of the present knowledge and to add further information regarding the positions involved and the yields obtained in the metalation of various compounds.

Since different solvents were to be employed for the metalations, some attention was given to the preparation of organometallic compounds in various media. *n*-Butyllithium was prepared from *n*-butyl chloride and lithium in benzene, pentane, hexane, butyl ether, and dioxane. The yield of *n*-butyllithium in each case as determined by titration represented approximately 65-70 per cent of the theoretical value. Tri-*n*-butylamine, however, proved unsatisfactory as a solvent for the same preparation. The preparation of phenylsodium, phenylpotassium, and phenylenedisodium was investigated in various solvents.

Dibenzothiophene was subjected to the action of *n*-butyllithium in ethyl ether, benzene (at 80°), pentane (at 35°), hexane (at 65°), dioxane (at 30°), butyl ether (at 30° and 80°). The yield of metalation product was negligible in all solvents but ethyl and butyl ether. When dibenzothiophene was treated with *n*-butyllithium in butyl ether at 30° for twenty hours, a 61 per cent yield of 4-dibenzothiophenecarboxylic acid was obtained on treatment with carbon dioxide, while the same reaction, when heated to 80° for twenty hours, gave a 90 per cent yield of the same acid. When dibenzothiophene was treated with *n*-amylsodium prepared in petroleum ether according to the directions of Morton³, a 37 per cent yield of 4-dibenzothiophenecarboxylic acid was obtained.

The metalation of bibenzyl was studied using as metalating agents *n*-butyllithium in ether, *n*-butylsodium in tributylamine, and *n*-butylpotassium in benzene. After the reaction products had been carbonated, the acids were isolated and identified. Metalation with *n*-butylsodium and *n*-butylpotassium yielded α , β -diphenylsuccinic acid (30 per cent and 52 per cent, respectively). *n*-Butyllithium, however, gave a poor yield (0-1 per cent) of *p*-(β -phenylethyl)-benzoic acid.

To study the metalation of substituted methane derivatives, phenyl-*p*-tolylmethane and phenyl- α -naphthylmethane were treated with *n*-butyllithium for thirty-six and twenty-four hours, respectively. Carbonation formed 49 per cent of phenyl-*p*-tolylacetic acid and 80 per cent of phenyl-

¹ Original thesis submitted June, 1938. Doctoral thesis number 478.

² Gilman and Young, *J. Am. Chem. Soc.*, 56:1415. 1934.

³ Morton and Hechenbleikner, *J. Am. Chem. Soc.*, 58:2599. 1936.

α -naphthylacetic acid. Observing the increasing yield of substituted acetic acids obtained from these compounds, it was suggested that a group of similarly substituted derivatives of methane would be a satisfactory series to study to determine the effect of variations in a portion of a molecule upon the extent of metalation.

To continue the investigation of compounds containing an active methylene group, 9,10-dihydroanthracene was treated with an ether solution of *n*-butyllithium for twenty hours giving, on carbonation, 80 per cent of 9,10-dihydro-9-anthracenecarboxylic acid and 8 per cent of 9,10-dihydro-9,10-anthracenedicarboxylic acid.

When biphenyl was treated with *n*-butyllithium in ether, the yield of acidic material represented 7-15 per cent of the theoretical value, being identified as a mixture of *o*- and *p*-phenylbenzoic acids. In benzene, however, *n*-butyllithium did not cause the metalation of the nucleus. Methyl-lithium in butyl ether proved valueless due to the rapid cleavage of the ether, while the low yield (5 per cent) of acid obtained after *n*-butyl-sodium had reacted upon biphenyl in petroleum ether indicated the inapplicability of that solvent for metalations.

In the metalation of naphthalene by *n*-butyllithium, a mixture of α - and β -naphthoic acids was obtained. The amount of acidic material varied with the time of heating the reaction, amounting to 13 per cent in fifteen hours and 20 per cent in thirty hours. Phenylcalcium iodide did not cause metalation of the nucleus. *n*-Butylsodium in tributylamine yielded 28 per cent of the acid mixture after thirty-six hours of refluxing.

In an attempted metalation of phenanthrene by *n*-butyllithium, the trace of acid obtained was identified as 9-fluorenicarboxylic acid. The probable source of this acid was the fluorene present as an impurity in the starting material⁴. The phenanthrene nucleus did not undergo metalation.

Furan and 2-methylfuran were treated with phenyllithium to yield, after carbonation, 38 per cent and 17 per cent of furoic and 5-methyl-2-furoic acid, respectively. 2,5-Dimethylfuran, under the same conditions, gave an unstable acid which evolved carbon dioxide upon acidification.

Since all compounds containing an ether linkage had undergone metalation by organoalkali compounds only in the position ortho to the ether bridge, more ethers were investigated in a search for a broad generalization. As a result of the metalation of ethers, it was suggested that the metalation of compounds containing an ether linkage might serve as a method for the introduction of substituents into the position ortho to the oxygen bridge.

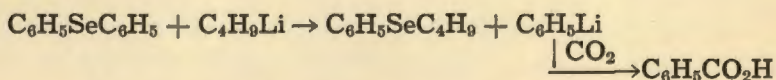
2-Methoxydibenzofuran was metalated by *n*-butyllithium to yield, after carbonation, 60 per cent of a mixture of 2-methoxydibenzofurancarboxylic acids. The mixture contained approximately three parts of 2-methoxy-1-dibenzofurancarboxylic acid to one part of 2-methoxy-3-dibenzofurancarboxylic acid.

After treatment with *n*-butyllithium and subsequent carbonation, anisole yielded 19 per cent of *o*-methoxybenzoic acid and 40 per cent of di-*o*-anisyl ketone. The high yield of ketone demonstrated the disadvantage of employing carbon dioxide as a reagent for the study of the products of a metalation involving an organolithium compound. When phenyl-

⁴ Jeanes and Adams, *J. Am. Chem. Soc.*, 59:2608. 1937.

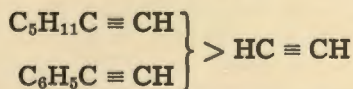
sodium in benzene was employed, 44 per cent of the same acid was obtained after 24 hours and 64 per cent after 48 hours.

The metalation of phenyl ether and its analogs (phenyl sulfide and phenyl selenide) was effected with *n*-butyllithium. After six hours, 54 per cent of *o*-phenoxybenzoic acid and 24 per cent of *o*-phenylmercaptobenzoic acid were obtained. When the time was increased to twenty hours, yields of 60 per cent of *o*-phenoxybenzoic acid and 30 per cent of *o*-phenylmercaptobenzoic acid showed that an increase in metalation occurred on increased time of reaction. Some cleavage of phenyl sulfide was evident from the odor of thiophenol, which was present only after the longer period of heating. When phenylsodium, suspended in benzene, served as the metalating agent, 56 per cent of *o*-phenylmercaptobenzoic acid was obtained. The odor of thiophenol was practically absent in this run. Phenyl selenide did not give a metalation product but was cleaved to butyl phenyl selenide and benzoic acid, which indicated the course of reaction as:



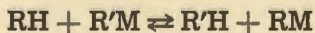
It was predicted that the cleavage of phenyl telluride would be still greater. Possibly this tendency of compounds to display increasing instability toward metalating agents with decreasing tendency to undergo metalation might be extended from Group VI to other groups of the periodic table.

Ethynylsodium in liquid ammonia was treated with 1-heptyne and phenylacetylene. Reaction occurred in both cases to form heptynylsodium and phenylethynylsodium, respectively. The organometallic compound was identified by adding benzophenone and isolating the corresponding carbinol. In regard to the relative acidity of the three compounds, they lie in the following order, with the hydrogen in phenylacetylene probably more acidic than that of 1-heptyne.



In an effort to correlate acidity with metalation, the radicals attached to hydrogen were placed upon a parabolic curve symmetrical to the positive vertical axis so that reading along the vertical axis from top to bottom showed the decreasing tendency of the compounds to undergo metalation.

The radicals were assigned positions upon the curve such that any radical when attached to hydrogen (RH) would react with the organometallic compound (R'M) of any radical below it on either side of the parabola.



By means of this curve, it may be possible to predict yields in the metalation of compounds from a knowledge of the relative lability of the radical attached to the acidic hydrogen, or *vice versa*, to predict relative lability from the yields of metalation products.

THE ECOLOGY AND MANAGEMENT OF THE BLUE-WINGED TEAL (*QUERQUEDULA DISCORS* (LINNAEUS))¹

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Received June 25, 1938

The purpose of this investigation was to correlate the life habits and requirements of the Blue-winged Teal [*Querquedula discors* (Linnaeus)] with the effects of its present agricultural environment in the hope of developing a more substantial niche for the bird in Iowa. The research was initiated July 1, 1932 and continued until February 1, 1937. The greater part of the observations were made in Clay, Palo Alto, Dickinson, and Emmet Counties, Iowa. Supplementary observations were made in Minnesota, Nebraska, South Dakota, North Dakota, Manitoba, and Mexico.

The Blue-winged Teal is known by at least 47 vernacular names over its breeding, migratory, and wintering ranges. The sex ratio of 5,090 birds averaged 59 per cent males and 41 per cent females.

The breeding range of the bird was segregated into five types. They are named as follows in the order of their importance: true prairie area; mixed prairie area; boreal forest area; deciduous forest area; and lake forest area.

Fall migration began each year in Iowa about August 15 and usually was completed by November 1. The major portion of the flight occurred in the Mississippi Valley and Central Flyways. Shooting seasons in the northern states added little if any impetus to the fall migration.

The wintering grounds of the teal extend over thousands of square miles in Mexico, Central America, South America, and the southern part of the United States. It was determined that at least 95 per cent of the Blue-winged Teal winter south of the southern border of the United States. The southernmost record for the bird is Ovalle, Chile (Schalow, 1898). The birds were shot for sport and market purposes on the wintering grounds observed in Mexico.

Early in January from the wintering grounds the birds started northward on the spring flight. With few exceptions, the flyways over which they passed were those used during the fall migration. The main flight passed through the northern states between April 1 and April 30 each year.

Courtship began on the wintering grounds late in December and early January. Courtship became evident about the same time that the nuptial plumage of males became apparent and when the birds were getting started on the spring flight. By the end of March, when the first birds reached Iowa, the process of courtship had become a daily affair. The climax of courtship and mating was reached just prior to the nesting season.

The average distance of nests from water was 41.5 yards. It was found that 95.6 per cent of the nests were located within 220 yards of the shore-

¹ Original thesis submitted June 25, 1937. Doctoral thesis number 422.

line. The average elevation of nests above the marsh level was 2.4 feet. The average number of eggs found in 341 nests was 9.3 per nest.

The ducklings hatched 21 to 23 days after incubation began. So far as could be determined the young were led by the mother to water within twelve hours after hatching.

Of 223 nests, 40.4 per cent were destroyed by adverse weather conditions, agricultural practices, predators, and other causes. Only 14.8 per cent of twenty-seven discovered renesting attempts were successful. The number of young that reached the migratory stage was 5.1 per successful nesting female.

Bluegrass (*Poa pratensis*), the most common type of nesting cover, constituted the cover of 160, 47 per cent, nests. One hundred and thirty-two nests, 38.8 per cent, were found in slough grass (*Spartina Michauxiana*). Forty-eight nests, 14 per cent, were found in alfalfa (*Medicago sativa*). The bluegrass and slough grass often had other grasses and forbs mixed in with them.

The presence of ducklings in the respective types of rearing cover combinations indicated the value of such plant associations for rearing habitats. The rearing plant combinations are listed as follows in the order of their importance: great bulrush (*Scirpus validus*), round bulrush (*S. occidentalis*), and river bulrush (*S. fluviatilis*) associates; river bulrush, great bulrush, and bur-reed (*Sparganium eurycarpum*) associates; river bulrush and cat-tail (*Typha latifolia*) associates; river bulrush and sedge (*Carex riparia*) associates; reed (*Phragmites communis*) and cat-tail associates; and, bur-reed, sweet flag (*Acorus Calamus*), and larger blue flag (*Iris versicolor*) associates.

Approximately 75 per cent of the food found in 358 stomachs consisted of plant material. The four plant families, Cyperaceae, Najadaceae, Gramineae, and Polygonaceae were by far the most common groups of plants represented in the stomachs. Animal food constituted about 25 per cent of the food eaten by the Blue-winged Teal. Of the invertebrate foods eaten, insects and molluscs out-numbered other forms. The vertebrates were represented by fishes found in five stomachs.

The proper amount of grazing by cattle on marsh margins to insure permanent pasture is conducive to some teal nesting. Many areas, particularly inlets to water areas, should be revegetated to curtail soil erosion and in so doing would provide hay for live stock and nesting cover for teal, pheasants, and other wildlife.

The prairie habitat seemed to furnish the best breeding conditions for the Blue-winged Teal. The timbered tracts had but little nesting cover for teal and in addition supported a number of animals that are known to be predators of nesting ducks and their young. Not one successful nest was found in cover beneath trees or shrubs.

It was found that 95.6 per cent of the puddle ducks nested within 220 yards of the shoreline of a marsh, slough, or pot-hole regardless of the size of such a water area. Thus, the small water areas as a whole were conducive to greater duck production.

By sampling a lake region and obtaining data concerning nest densities, nest destruction, successful nests, and juvenile survival a fairly accurate census can be made to determine the year's production.

The development of appreciation of our wildlife resources the past few years in the United States seems to lead toward a permanent pro-

gram for the perpetuation and increase of many wildlife species. Correct land use practices, water conservation, soil conservation, and the economic evaluation of sporting and non-sporting species have all aided and will continue to better the environment for the Blue-winged Teal.

LITERATURE CITED

SCHALOW, HERMAN

1898. Die Vögel der Sammlung Plate. Zool. Jahrb., Supplementband. 4:641-749 (Fauna Chilensis, v. 1).

A CRITICAL STUDY OF THE PRECISION AND VALUE OF HALOGEN ADDITION REACTIONS APPLIED TO DAIRY RESEARCH¹

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Received June 25, 1938

In order to determine the importance of the quantity of free unsaturated fatty acids in butter fat, a precise semi-micro iodine number method was needed. When this work was initiated (1932), no semi-micro iodine number method had been described in the literature. It seemed logical, therefore, that a standard method should be adapted for use in this study.

Preliminary results indicated that the reaction of the Hanus (1) reagent was especially sensitive to variations in sample weight. Hübl iodine numbers calculated with the Schmidt-Nielsen and Owe (4) formula, did not depend greatly upon the weight of sample but the age and acidity of the reagent were important factors.

The following halogenating reagents (prepared according to the directions given in the literature cited) were studied on a semi-micro scale for butter fat and insoluble butter acids: Hübl (4), Wijs (1), Hanus (1), Rosenmund and Kuhnhehn (3) and Kaufmann (2). Iodine number determinations were made with each of these reagents on a sample of butter fat and the insoluble acids obtained from it at varying reaction periods up to one week. This study also included the Hübl reagent in which 95 per cent ethyl alcohol was replaced by absolute methyl alcohol. The reactions were carried out in the dark at $25^{\circ}\text{C.} \pm 0.4$.

Iodine numbers were plotted against reaction periods for each of the above reagents. If equilibria were attained, there should be flat sections (zero slopes) in the curves. The curve obtained with the Kaufmann reagent and butter fat showed but little slope from 30 to 168 hours and with the insoluble acids from 70 to 168 hours. The Hübl reagent gave similar curves when the values were calculated according to the Schmidt-Nielsen and Owe formula, but when the average of the beginning and final blanks was used for the iodine number calculations, no flat sections in the curves were evident. Both the Kaufmann and Hübl reagents gave higher values for the fat than for the acids; this anomalous behavior can not be explained satisfactorily.

None of the other curves seemed to show any indication towards a zero slope when plotted on such a scale as to include reaction periods up to one week, but the initial values of fat and acids were in a reasonable relationship to each other. When the portions of these graphs representing the reaction on butter fat from 0 to 12 hours were plotted on a much larger scale, the Kaufmann, Hanus and Rosenmund and Kuhnhehn curves approached zero slopes for variable periods of reaction. The Wijs and Hanus curves were similar although the slopes were somewhat greater with the Wijs reagent.

The iodine numbers obtained with the Rosenmund and Kuhnhehn

¹ Original thesis submitted March, 1938. Doctoral thesis number 456.

reagent on butter fat increased only about 0.3 unit from 30 min. reaction period to 5 hours. None of the other methods gave values as nearly constant as these over a reaction period of this length. It was considered that these results indicated that further study was warranted with the Rosenmund and Kuhnhehn reagent.

As a result of this study the following conditions were worked out for its use: Weigh from 10 to 100 mg. of butter fat or insoluble butter acids into a 125 ml. iodine flask and dissolve in 2 ml. of carbon tetrachloride. Add 5 ml. of 0.1N pyridine sulfate dibromide in acetic acid freed of reducing compounds, and close the flask with a glass stopper on the surface of which one drop of syrupy phosphoric acid was spread to prevent escape of reagents. Rotate the contents of the flask a few times and allow the reaction to take place in the dark at 25° C. for 4 hours. After the reaction period, add 2 ml. of 10 per cent potassium iodide to the rim of the flask, cautiously turn the stopper in order that small gas bubbles will go through the potassium iodide. Thoroughly mix the potassium iodide with the reactants and then add 25 ml. of oxygen-free water and mix well. Titrate the iodine with sodium thiosulfate (approximately 0.011N) to the complete disappearance of the yellow color (a slight excess of thiosulfate). Stopper the flask and shake violently, rinse liquid adhering to stopper into the flask and add 4 to 5 ml. of 0.5 per cent starch indicator. Back titrate the excess of sodium thiosulfate with iodine in potassium iodide (approximately 0.005N) from a burette graduated to 0.02 ml.

When sample weights are approximately alike, the semi-micro Rosenmund and Kuhnhehn method yields replicate determinations which agree within 0.3 of an iodine unit.

It was considered that as final checks on the semi-micro Rosenmund and Kuhnhehn method the following factors should be studied: 1. effect of excess reagent on the iodine number obtained for butter fat and its insoluble acids, 2. agreement of replicate determinations on weighed samples, 3. comparison of the value by this method with the value obtained with a carbon tetrachloride solution of iodine monobromide conducted in such fashion that substitution should be detected and 4. comparison of the value by this method with that obtained by the bromine vapor method.

It is considered from these studies that excess reagent from 14 to 90 per cent will cause a variation of 1.0 unit in the iodine number of butter fat, while variation of excess reagent from 20 to 90 per cent will cause a variation of approximately 0.5 unit with the insoluble acids from butter fat.

Replicate determinations on weighed samples should check within 0.5 unit.

The attempted determination of a "true" iodine number by measuring substitution and correcting the iodine number for it did not prove satisfactory nor did it afford the desired comparison.

The comparison with the bromine vapor method which should give values very closely representing only the degree of unsaturation of butter fat indicate that for this fat the Rosenmund and Kuhnhehn method, used as described above, gives very nearly the correct iodine number.

The bromine vapor method was studied with regard to its adaptation for use with butter fat and its acids. Further study is necessary to determine the precision of this method.

LITERATURE CITED

1. Association of Official Agricultural Chemists
1936. Official and tentative methods of analysis, p. 410-412. 4th ed. Washington, D. C.
2. KAUFMANN, H. P.
1935. Studien auf dem Fettgebiet. p. 23-24. Maurer and Dimmick, Berlin.
3. ROSENMUND, K. W., AND W. KUHNHENN
1923. Eine neue Methode zur Jodzahlbestimmung in Fetten und Ölen unter Verwendung von Pyridinsulfatdibromid. Z. Nahr. Genussm. 46:154-159.
4. SCHMIDT-NIELSEN, S., AND A. W. OWE
1923. Die Bestimmung der Jodzahl. I. Vergleichende Untersuchungen über die Jodzahl der Fette. Skrifter Videnskapsselskapet. I. Kristiania. Mat. natur. Klasse. No. 15.

SOME FACTORS AFFECTING THE TIME OF SOLUTION OF DEXTROSE¹

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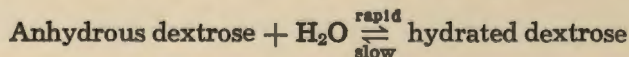
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Dextrose² is quite soluble even in cold water, however, the time required to prepare solutions within five per cent of a saturated solution at a given temperature is too long for the practical preparation of syrups. Because of the increased consumption of purified dextrose as a constituent in food preparations the problem of solubility time has become an important one. It was the purpose of the present investigation to determine the more important factors affecting the solution time of dextrose.

Sandera³ has summarized various methods for determining the solution time of sugars, no one method of which is applicable in commercial practices. A method was developed, therefore, which was adaptable to large scale preparations. The apparatus used consisted of a series of Soxhlet extraction flasks, each with a separate stirring unit, arranged in a black lined constant temperature bath controlled by a rheostat sensitive to 0.05°C. The sample being tested for solution time was placed in one of the flasks and stirred at the desired temperature until solution was complete. By means of a strong light source directed into the flask against the black background it was possible to check duplicate runs within two per cent even when the method was adapted to runs using one hundred pounds of dextrose. In order to obtain consistent results it was necessary to prevent settling out of sugar crystals by control of the stirring rate, shape of container, size of dextrose crystals and rate of addition of the dextrose.

At all concentrations the solution time of hydrated dextrose increased regularly with a temperature decrease, whereas in the case of anhydrous dextrose a pronounced increase in solution time was observed at definite temperatures and concentrations. In general the time for complete solution of anhydrous dextrose was ten minutes or less when the percentage concentration was not more than one-half the numerical value of the temperature in Fahrenheit degrees.

Initial concentrations of anhydrous dextrose were ten to fifteen per cent higher than those of the hydrated form. Rapid crystallization of hydrated dextrose occurred in the more concentrated initial solutions of anhydrous dextrose, thus proving the higher solubility of the anhydrous form as well as the rapid conversion of anhydrous to hydrated dextrose in solution. Conversion of hydrated to anhydrous dextrose is a slow reaction as exemplified by the slow solubility of the hydrated form.



¹ Original thesis submitted March, 1938. Doctoral thesis number 460.

² In this abstract the term "dextrose" refers to the alpha-form of 4-glucose except where otherwise stated.

³ Sandera, Z. Zuckerind. Czechoslovak. Rep., 61:275. 1937.

The equilibrium mixture after dextrose hydrate crystallization consisted of approximately fifteen per cent anhydrous and eighty-five per cent hydrated dextrose.

A number of compounds yielding basic solutions when dissolved in water were tested for their catalytic effect upon the dissolution of dextrose. The pH values for these solutions, both before and after adding the dextrose, were determined by means of a Coleman electrometer having a glass electrode.

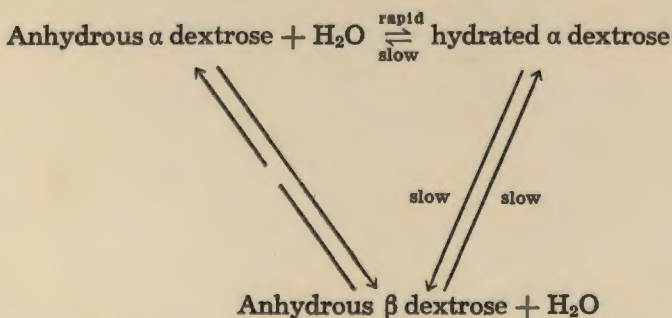
In solutions of the alkaline hydroxides, including LiOH, NaOH, KOH, Ca(OH)₂, Sr(OH)₂ and Ba(OH)₂, the solution time of dextrose was a direct function of pH or normality of reagent solution. Time of solution, in other reagents, however, was not determined by pH, but was dependent upon the nature of the components of the reagent solution. There was no difference in the catalytic effect among the metallic ions in any salt series.

Approximately twice the catalytic effect was shown by the sodium or potassium salt of maleic acid as by that of its geometric isomer, fumaric acid. Aqueous solutions of organic bases such as pyridine or quinoline had little influence on the solution time of dextrose. The methyl and ethyl substituted ammonium hydroxides decreased in their catalytic effect as the weight of the molecule was increased. Soluble sodium silicates were effective in direct proportion to the sodium oxide-silicon oxide ratio.

In a buffered mixture, such as the solution resulting from the titration of a phosphoric acid solution with sodium or potassium hydroxide, a maximum solution time resulted at a pH of approximately 3.8.

Catalysts of mutarotation were, in general, found to be correspondingly efficient as solution catalysts. Reagents which catalyzed mutarotation to the extent that equilibrium between the alpha- and beta-forms was established within fifteen minutes caused the alpha-dextrose to dissolve within the same period of time. Beta-dextrose as well as mixtures of alpha- and beta-dextrose containing as much as seventy-five per cent of the alpha-form dissolved almost instantaneously, whereas an equal concentration of the alpha-form alone required three hours for complete solution.

In accordance with the data presented the following equilibrium depicts the condition during the dissolution of dextrose:



Any agent or reagent which tends to produce a more soluble form or condition of dextrose will accelerate its solution.

HIGH FREQUENCY GLOW DISCHARGE WITH INTERNAL ELECTRODES¹

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High frequency electric discharges in hydrogen were excited by voltages applied to a discharge tube by means of internal carbon electrodes. The intensities of certain lines in the hydrogen spectrum were studied quantitatively as a function of frequency of excitation and pressure.

The high frequency potential was supplied by a push-pull oscillator which used 100 watt No. 852-type tubes. The potential difference applied between the electrodes was measured by a cathode ray oscillograph and maintained at a constant peak potential of 1500 volts.

The gas system was made entirely of pyrex except for a quartz window connected by a graded seal to one end of the discharge tube and a palladium tube similarly connected to a side tube. An oil diffusion pump that was air-cooled and of the vertical stream type charged with Apeizon oil B which was heated electrically and a Hyvac pump made up of the evacuating system. A liquid air trap was used to condense all impurities that could be frozen out. The pressure was measured by a pirani gauge. Hydrogen was admitted to the discharge tube by diffusion through palladium.

The discharge tube was 50 cm. long and 4.8 cm. in diameter, and each end was drawn to 2.5 cm. by means of shoulder seals. The tube was baked-out for several hours while on the pumps in an especially built electric furnace at a temperature between 450° and 500°C. Several times during the process the tube was flushed with hydrogen.

Spectrograms of the discharge were taken with a Bausch and Lomb medium quartz spectrograph in conjunction with a logarithmic sector disc. This type of disc gives spectral lines whose lengths are proportional to their intensity. W. and W. panchromatic plates of the same emulsion batch were used and were developed with developer D-19 from the same stock solution under the same conditions of temperature and time.

The data were obtained for pressures from 5 to 300 microns and for frequencies of excitation from 0 to $5 \cdot 10^7$ C. P. S. Intensity vs. wavelength curves were plotted for different pressures. An explanation of the maxima and minima which occur in the curves and the optimum pressure was given on the basis of J. Thomson's (2) theory of sparking potentials.

The results of the investigation show:

1. There is a variation in intensity of the spectrum lines for different pressures and frequencies of excitation.
2. The results are very similar when either internal or external electrodes are used.

¹ Original thesis submitted July, 1937. Doctoral thesis number 442.

3. The ratios of the intensities of H_β , H_γ and H_δ to H_α increase for low pressures.

4. The ratios of the populations in the higher order states increase with a decrease in pressure.

5. For low pressures the probability of excitation is as great for the higher frequency terms as for the lower ones.

6. Thomson's theory for sparking potentials applies to maintenance potentials.

7. The optimum pressure is such that approximately 12 times the kinetic theory mean free path of the electron is equal to the interelectrode distance.

8. The amplitudes of oscillation of the free electron for wavelengths which give line intensity minima and maxima are multiples of the electronic mean free path.

9. The impurities which are commonly observed in this type of discharge phenomena were eliminated by the design of the tube and the use of liquid air.

10. The most striking observation is the similarity of the intensity vs. wavelength curves to those obtained by Bachman (1).

BIBLIOGRAPHY

1. BACHMAN, CHARLES
1935. Effect of high frequency excitation upon intensities of spectral lines. Unpublished Thesis. Library, Iowa State College, Ames, Iowa.
2. THOMSON, JOHN
1937. Sparking potentials at ultra-high frequencies. Phil. Mag. ser. 7. 23:1-24.

STUDIES ON CHLORINE DISINFECTION¹

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Chlorine compounds having disinfectant properties have been used for over a century and their consumption today probably surpasses that of all other disinfectants combined. They are employed as sterilizers, for instance, in the maintenance of safe water supplies, in medical practice, in many food industries, in the chlorination of sewage and sewage disposal-plant effluents, and in the control of slime formation in pulp and paper mills.

Originally used in the form of sodium and calcium hypochlorites, chlorine has more recently been employed in the form of chlorine gas in water solution, and in various types of compounds generally known as "chloramines," which possess an :N-Cl linkage.

The methods of estimating the strength of chlorine disinfectants are the same as those employed in analyzing hypochlorite bleaching solutions. The original term "available chlorine" has been retained although there are objections to its use.

A review of the literature reveals a lack of agreement in regard to the relative germicidal power of chlorine compounds and the manner in which they exert germicidal action. It was therefore decided to investigate various aspects of the chlorination process, particularly the relative germicidal activity of the several forms of chlorine compounds, and to note the effect of alterations in reaction (pH), temperature and concentration upon disinfection efficiency.

Bacterial spores were found to be particularly suitable and applicable for the objectives of this study. The culture employed had been used previously in studies on disinfection with alkalies and the organism, *Bacillus metiens* (nov. sp.), was further described. A dried spore suspension in powdered lactose was employed. No appreciable changes in numbers of viable cells per unit weight were observed and the spores did not exhibit detectable changes in resistance to chloramine-T during storage, for a period of over 6 months. Those observations suggested that the dried spores were not undergoing any physiological changes. The dried spores, when suspended in distilled water and connected to a Barcroft-Warburg manometer, did not reveal any evidence of respiration. Spore suspensions in distilled water showed no appreciable diminution in numbers after several weeks exposure at room temperature.

The germicidal efficiencies of chlorine disinfectants were found to be markedly affected by temperature, concentration, and reaction (pH) alterations. The results of the investigation are of interest in that they give useful information regarding the relative germicidal power of chlorine compounds and they account for certain of the conflicting opinions previously reported. The germicidal activity of chloramine-T solutions was found to be affected as follows:

¹ Original Thesis submitted December, 1933. Doctoral thesis number 260.

(a). The reaction (pH) of the disinfecting solution markedly influenced the killing time, the germicidal efficiency increasing with increasing acidities. For example, a solution with 2,000 p.p.m. available chlorine, at an initial reaction of pH 8.7, gave a killing time of 64 hrs., whereas at pH 6.0 the killing time was only 5.4 hrs.

(b). Doubling the concentration resulted in a reduction of the killing time to approximately one-half.

(c). Rises of 10° C. in temperature (in the range 25° to 55° C.) resulted in a reduction of the killing time by approximately 71 per cent when employing solutions with an initial reaction of pH 8.7. The same temperature change with solutions at pH 6.0 resulted in killing-time reductions of about 82 per cent. These temperature effects were approximately uniform up to 55° C. for a given solution and reaction.

Concerning the disinfecting action of hypochlorites it was found that:

(a). The reaction (pH) markedly affected germicidal properties which were increased as the acidity rose. For example, with 1,000 p.p.m. available chlorine at a reaction of pH 11.3, the killing time was 64 min., whereas at pH 7.3 the killing time was less than 20 sec.

(b). In solutions of two commercial calcium-hypochlorite preparations employed, the "available chlorine" was not a measure of the germicidal power (even with various concentrations of the same preparation). For example, the killing times obtained with 1,000 and 100 p.p.m. available chlorine of the same compound were not appreciably different. This apparently anomalous result is explained by the fact that the more dilute solution was less alkaline (due to dilution of the stabilizing agent) with resultant increased germicidal power which was found to be approximately proportional to the calculated amount of hypochlorous acid present.

(c). From a consideration of the survivor curves, additional evidence was obtained to support the contention that the concentration of undissociated hypochlorous acid was probably the determining factor in the rate of disinfection by hypochlorites.

Oxidation potentials of chloramine-T solutions were found to be unsatisfactory as measures of germicidal activity, since doubling the concentration reduced the killing time almost to one-half whereas the oxidation potential was not significantly altered.

Solutions of chloramine were strongly germicidal, and this germicidal power was less affected by pH changes than was the case with the hypochlorite and chloramine-T solutions.

Concerning the relative germicidal powers of the three types of chlorine compounds which were studied, in solutions practically free from organic matter, it was found that for a given concentration of available chlorine:

(a). Chloramine-T exhibited the weakest germicidal action.

(b). Monochloramine was more strongly germicidal than hypochlorites when the comparisons were made in highly alkaline solutions. In less alkaline solutions, at reactions below the range pH 9.5 to 10.0, hypochlorites were more germicidal than monochloramine or the resulting mixtures of monochloramine and dichloramine.

The nature of the survivor curves (log survivors against time) was not the same in disinfection with the various types of chlorine compounds studied. The survivor curves obtained in the experiments with monochloramine simulate a straight line and resemble those obtained with chloramine-T. The hypochlorite survivor curves show a great deviation from a straight line relationship, particularly at the higher alkalinities where a pronounced lag followed by a rapidly increasing death rate is noted. A fundamental difference in the mechanism of disinfection with the two general types of chlorine compounds is thereby indicated.

In disinfection with chloramine and chloramine-T hypochlorous acid does not appear to play the significant role which it exhibits with hypochlorites.

Disinfecting action for all of the compounds appears to be associated with the presence of a positive chlorine atom.

AMINO AND HYDROXY DERIVATIVES OF DIBENZOFURAN¹

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Received June 25, 1938

Orientation studies have disclosed that all of the positions in the dibenzofuran nucleus, with the exception of the 1- and 9-positions, are vulnerable to direct nuclear substitution. An inspection of the complex morphine structure impresses one with the necessity of being able to substitute the (so-called critical) 1-, 4-, 6-, and 9-positions of dibenzofuran in order to synthetically approach the constitution of this important alkaloid.

The objectives of the orientation and metalation studies of this investigation were to render 4,6-disubstituted dibenzofurans more available, to prepare certain 1,4,6,9-tetra-substituted dibenzofurans, and to explore the feasibility of synthesizing 4,5-phenanthrylene oxide and 4,5-phenanthridine oxide derivatives through cyclization methods applied to the 1- and 9-positions of 4,6-disubstituted dibenzofuran derivatives, that is, 4,6-dimethoxydibenzofuran. Amino and hydroxy derivatives were chosen because of their inherent ability to activate the aromatic nucleus. The compounds were also to be submitted for pharmacological test, and a review of useful analgesics, coupled with the results of previous physiological tests on dibenzofurans, favored the conclusion that amino and hydroxy derivatives were most apt to manifest analgesic activity.

An attempt has been made to tabulate all amino and hydroxy derivatives of dibenzofuran which have appeared in the literature prior to May, 1938. In addition, a number of compounds taken from unpublished work in this laboratory have been included along with the new compounds described in the thesis. All available references to each individual compound have been collected. Because of the inordinate task of compilation, the brazans, the dinaphthylene oxides, the morphine alkaloids, and many other derivatives of fused ring systems inhering a dibenzofuran nucleus have been omitted. *Chemical Abstracts* has been the criterion in deciding which compounds should be included.

The likelihood of linking together the 1- and 9-positions of dibenzofuran by means of a two-carbon bridge for the synthesis of 4,5-phenanthrylene oxides finds the support of analogy in the literature. Pertinent literature citations to analogous cyclizations have been presented.

The preparations of the following amino and hydroxy derivatives of dibenzofuran have been described: 4,6-dihydroxydibenzofuran (m.p. 200-202°), 3-hydrazinodibenzofuran (m.p. 174-175°), 1,2,3,4-tetrahydro-6-aminodibenzofuran hydrochloride (m.p. 227-228° decompn.), bi-(4-dibenzofuryl) (m.p. 191°), di-(methoxy-1-dibenzofuryl) ketone (m.p. 234°), bi-(4-methoxy-1-dibenzofuroyl) (m.p. 329°), bi-(4,6-dimethoxy-1-dibenzofuroyl) (m.p. above 300°), 1-chloroacetyl-4-methoxydibenzofuran (m.p. 165-166°), 1-ethoxalyl-4-methoxydibenzofuran (m.p. 113°), 4-methoxy-1-dibenzofuryl- α -oxoacetic acid (m.p. 187°), 4-methoxy-diben-

¹ Original thesis submitted June, 1938. Doctoral thesis number 474.

zofuryl- α -oxoacetic acid semicarbazone (m.p. 211-212° decompn.), 3-hydroxy-4-methoxydibenzofuran (m.p. 109-110°), bi-(6-methoxy-4-dibenzofuryl) (m.p. 237-238°), bi-(6-hydroxy-4-dibenzofuryl) (m.p. 285-286°), 3,4-dimethoxydibenzofuran (m.p. 60-61°), 3,4-dihydroxydibenzofuran (m.p. 164-164.5°), 4,6-dimethoxydibenzofuran (m.p. 128-129°).

4,6-Dimethoxydibenzofuran picrate (m.p. 161-162°), 4-bromo-6-methoxydibenzofuran (m.p. 114°), 4-bromo-6-hydroxydibenzofuran (m.p. 138-139°), 4-amino-6-methoxydibenzofuran (m.p. 109°), 4-amino-6-hydroxydibenzofuran (m.p. 191.5-192.5°), 1-bromo-3,4-dimethoxydibenzofuran (m.p. 108°), 1-bromo-3-hydroxy-4-methoxydibenzofuran (m.p. 161-162°), 1-bromo-4,6-dimethoxydibenzofuran (m.p. 152°), 1,9-dibromo-4,6-dimethoxydibenzofuran (m.p. 167-168°), 1,9-dibromo-4,6-dihydroxydibenzofuran (m.p. 239-240°), 1,3(?) -dibromo-4-hydroxy-6-methoxydibenzofuran (m.p. 177-178°), 1,3(?) -dibromo-4,6-dimethoxydibenzofuran (m.p. 173.5-174°), 4,6-diaminobenzofuran (m.p. 152°), 4,6-diaminodibenzofuran picrate (m.p. 213° decompn.), 4,6-diacetaminodibenzofuran (m.p. 297-298°), 4,6-diacetoxydibenzofuran (m.p. 177°), 3,4-diacetoxydibenzofuran (m.p. 104-105°).

1-Acetyl-4,6-dimethoxydibenzofuran (m.p. 178-179.5°), 1-acetyl-3,4-dimethoxydibenzofuran (m.p. 90.5-91°), 1-acetyl-4,6-dimethoxydibenzofuran oxime (m.p. 203-204°), 1-acetyl-3,4-dimethoxydibenzofuran oxime (m.p. 156-157°), 1-acetamino-4,6-dimethoxydibenzofuran (m.p. 244-245°), 1-acetamino-3,4-dimethoxydibenzofuran (m.p. 196-196.5°), 1-benzeneazo-4-hydroxy-6-methoxydibenzofuran (m.p. 175°), 1-benzeneazo-4,6-dimethoxydibenzofuran (m.p. 170°), 1-amino-4,6-dimethoxydibenzofuran (m.p. 162-162.5°), 1-amino-3,4-dimethoxydibenzofuran (m.p. 162.5-163°), 4,6-dimethoxy-1-dibenzofurancarboxylic acid (m.p. 297-298°), methyl 4,6-dimethoxy-1-dibenzofurancarboxylate (m.p. 163°), diazo-methyl 4,6-dimethoxy-1-dibenzofuryl ketone (m.p. 151° decompn.), 4,6-dimethoxy-1-dibenzofurylacetamide (m.p. 210-211°), 4,6-dimethoxy-1-dibenzofurylacetic acid (m.p. 205-206°), 1,3,9-tribenzeneazo-4,6-dimethoxydibenzofuran (m.p. 191-193°) and di-(4,6-dimethoxy-1-dibenzofuryl) ketone (m.p. 254-255°).

Several attempts to prepare a phenanthrylene oxide derivative by intramolecular alkylation of 1-chloroacetyl-4-methoxydibenzofuran in intramolecular alkylation of 1-chloroacetyl-4,4-methoxydibenzofuran in the presence of aluminum chloride emphasized the noteworthy inertness of the chlorine atom under the conditions of the Friedel-Crafts reaction. In every case either starting material was recovered or hopeless decomposition took place.

The isolation of bi-(4-methoxy-1-dibenzofuroyl) from the Friedel-Crafts reaction of 4-methoxydibenzofuran with oxalyl chloride suggested that in order to realize the formation of the desired *o*-quinone with oxalyl chloride, both the 1- and 9-positions of the dibenzofuran nucleus should be activated by the introduction of suitable substituents in the 4- and 6-positions. Consequently, 4,6-dimethoxydibenzofuran was prepared and subjected to the Friedel-Crafts reaction. Again intermolecular condensation proceeded with the formation of bi-(4,6-dimethoxy-1-dibenzofuroyl) in excellent yield. Likewise, failure attended three efforts to synthesize a 4,5-phenanthridine oxide derivative from 1-aceta-

mino-4,6-dimethoxydibenzofuran by application of the Bischler-Napieralski reaction.

The synthesis of 4,6-dimethoxy-1-dibenzofurylacetic acid has supplied a compound which holds unprecedented promise of yielding a 4,5-phenanthrylene oxide derivative. One unsuccessful preliminary attempt to achieve ring closure has been described.

Because of the powerful *ortho*-orienting influence exerted by ether groups in organoalkali metalations, the metalation of 4-methoxydibenzofuran with *n*-butyllithium followed by subsequent oxidation of the product produced the two isomers, 3-hydroxy-4-methoxydibenzofuran and 4-hydroxy-6-methoxydibenzofuran in almost equal quantity. The unexpected isolation of bi-(6-methoxy-4-dibenzofuryl) from the same reaction product led to the discovery that considerable bi-(4-dibenzofuryl) is formed during the preparation of 4-hydroxydibenzofuran. A mechanism for this definitely established coupling reaction has been proposed.

THE GROSS ANATOMY OF *CORIZUS LATERALIS* (SAY) (HEMIPTERA, CORIZIDAE)¹

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Corizus lateralis (Say) is a small Hemipteron which occurs in many localities in the United States. It is found more easily on smart weed (*Polygonum pennsylvanicum* L.) than on any other plant. Because the insect is plentiful, readily kept in captivity, and because very little work has been done on the anatomy of the family Corizidae, the writer chose this species for a morphological subject.

Dissections were made after imbedding the insect just below the surface of beeswax in a Syracuse watch glass. This wax matrix held the small specimens firmly while they were being dissected. Boiling the insects for fifteen minutes hardened the internal organs to facilitate dissecting.

Micro-scalpels were improvised by breaking thin safety razor blades into small angular fragments, which were fastened to the tips of discarded dental scrapers by means of "Liquid Solder," a metallic cement. Micro-needles were made the same way, using "minuten" insect pins instead of the pieces of razor blades.

The n-butyl alcohol method proved satisfactory in making serial sections. Morty-micra sections were of more help than thinner ones for observing internal organs.

It was found that the wing veins could be observed very easily if the wings were mounted on a layer of glycerine jelly and covered with a cover glass so that the mounting medium was on only one surface of the wing.

An improvised microprojector was used in making most of the drawings. A compound microscope was placed horizontally, with a small focusing microscope lamp (six volts) as a source of concentrated brilliant illumination. A silvered prism at the ocular turned the rays down on the drawing paper. The size of the colored images could be changed by varying the projection distance.

Photomicrographs were made with the above described projection apparatus by using photographic bromide enlarging paper in lieu of drawing paper.

The anatomy of *Corizus lateralis* was found to be very complicated. The head is composed of inseparable sclerites. Two large compound eyes on each side of the head, two ocelli in the dorsal area between the eyes, and two six-jointed antennae constitute the more evident sensory structures of the head.

The beak is made up of a four-segmented labium which has a groove on its anterior surface to enclose the two mandibular and two maxillary bristles. These bristles interlock to form a food canal and a salivary canal running the length of the beak.

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The labial or salivary glands are located in the cephalic portion of the abdomen, with only two lobes resting in the thorax. Two salivary ducts connect the glands with the salivary syringe in the head.

There are no cervical sclerites in *Corizus lateralis*. The prothorax is very broad and large. The mesothorax and metathorax are combined into a pterothorax, bearing the four wings.

There is a pair of scent gland ostioles in the anterior pleural portions of the metathorax. The granular surrounding areas probably serve as evaporating plates.

The repugnatory or scent gland is located in the metathoracic sternum. Composed of two convoluted tubules, the gland rests between the sternal furca, with the reservoir almost covering both tubules. A valvular mechanism allows the secretion to be ejected from the reservoir.

Coxa, trochanter, femur, tibia, and tarsus compose the legs. The pretarsus is modified for locomotion by means of claws and pulvilli.

Basally, the fore wings are coriaceous with large veins, but the terminal portions are membranous. The hind wings are composed of three main regions—the remigium, vannus, and jugum. Vannal and jugal folds separate the wing areas. The fore and hind wings have a coupling device for uniting the two wings on each side into one functional unit.

Six segments of the abdomen are easily seen. The first is partially hidden by the metathorax. Dorsally, the fourth segment is concave at the base and at the apex, corresponding with the presence of a small dorsal scent gland. Segments seven and eight are usually retracted into the sixth and are modified for reproduction. The pleural regions form a prominent ridge or connexivum on each side of the abdomen, producing a trough-like tergum which is covered by the resting wings.

The alimentary canal is divided into the buccal cavity, crop, proventriculus, ventriculus, anterior intestine, posterior intestine, rectum, and anus. A stomodaeal valve is in the anterior part of the ventriculus, while a pyloric valve designates its caudal end.

Four malpighian tubules are connected with the anterior intestine. These tubules do not coalesce at their distal ends as they do in some Hemiptera. Rectal papillae are not present.

The heart is located in the dorsal section of the abdomen, with a slender aorta leading to the brain. Dorsal diaphragm muscles hold the heart in position. When viewed laterally, the ostia appear as tiny vertical elliptical perforations. Normally, the heart rate is eighty-five times per minute. Variations in temperature cause fluctuations in the number of pulsations. No reversal of flow was noticed. Numerous pericardial cells surround the heart. The aorta is located in the thorax and head, and is smaller than the heart, having no ostia or chambers.

The brain is divided into the protocerebrum, the deutocerebrum, and the tritocerebrum, which are not sharply constricted from each other.

Numerous nerves originate in the brain. A small frontal ganglion lies just in front. Short, thick, circumoesophageal connectives link the brain and the suboesophageal ganglion.

A single ganglion rests in the prothorax. The ganglia of the mesothorax and metathorax are combined to form a large pterothoracic ganglion. Because of a transverse constriction, this appears as two sep-

arate ganglia. Posteriorly, a large median nerve sends branches into the abdominal segments.

Tiny spindle-shaped ovarioles make up the ovaries. Anteriorly, the ovarioles taper into slender terminal filaments which end in the thorax. The ovariole ducts or pedicels unite to form two common lateral oviducts which fuse to produce the oviductus communis. An accessory gland is located on the floor of the sixth segment. Part of the spermathecal duct coils spirally around the spermatheca.

The testes are bright red and are composed of seven sperm tubes. They are divided into the germarium, zone of growth, maturation zone, and zone of transformation. Vasa deferentia connect the testes with the ejaculatory reservoir. A ductus ejaculatorium carries the spermatozoa from the reservoir to the penis.

EFFECT OF PLANT GROWTH-PROMOTING SUBSTANCES ON VITAMIN CONTENT AND REPRODUCTION OF LEMNA¹

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Plant growth-promoting substances of various kinds are formed in the shoots and roots of germinating corn (auxins) and in beef liver (pantothenic acid). These, together with related synthetic organic compounds, were used in inorganic media to determine their influence on the rate of reproduction of Lemna major grown in sterile cultures under electric light.

Vitamin B₁ has been shown to be a growth-promoting substance for excised roots. The production of vitamin B₁ in Lemna major, grown under varied conditions, was determined by rat-feeding experiments.

INFLUENCE OF EXTRACT OF BEEF LIVER (PANTOTHENIC ACID) ON GROWTH OF LEMNA

Williams (8) designated as "pantothenic acid" a growth-promoting factor for yeast which he prepared in a crude concentrated form by fractional electrolysis of an 80 per cent methyl alcohol extract of plant and animal tissue. The acid was concentrated in the acidic electrolyzed fractions and found to stimulate the growth of the yeast and also the growth of alfalfa seedlings (McBurney, 5). An 80 per cent methyl alcohol extract of beef liver, a material found by Williams (8) to contain pantothenic acid, was used as a source of this growth substance for experiments with Lemna. A water solution of the extract was concentrated by fractional electrolysis (Williams, 8).

Lemna, free from microorganisms, were grown in Clark's (3) sterile solution, with additions from the electrolyzed fractions of the liver extract, at a pH of 4.8. The plants were grown for four weeks under controlled environment (25° C. and 14½ hours exposure daily to mazda illumination of 150 foot-candles intensity), and were transferred to freshly prepared solutions at five day intervals (Clark, 2). The rate of reproduction of each culture was determined graphically by the method of Clark (3).

The rate of increase was stimulated by both the acidic and the alkaline fractions from the electrolyzed liver extract. Williams (8) did not report a marked stimulation of yeast from alkaline fractions but these gave a definite stimulation of the Lemna. McBurney (5) used only the acidic fractions for alfalfa seedlings and secured a stimulation of growth accompanied by a decrease in chlorophyll. The stimulation of Lemna was also accompanied by a loss of chlorophyll as well as a decrease in the size of fronds and inhibition of root growth.

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INFLUENCE OF AUXINS ON GROWTH OF LEMNA

The roots and shoots of germinated corn were used as a source of auxins, the plant growth-promoting substances discovered in the tips of oat coleoptiles by Went (7). Water solutions of a 95 per cent ethyl alcohol extract of the materials were concentrated by fractional electrolysis as before. Sterile Lemna were grown in inorganic media supplemented with the prepared extracts. The inorganic media and the growth conditions were identical with those outlined for the pantothenic acid experiments.

From the shoots of corn the auxins were not concentrated in any of the cells by the fractional electrolysis; from the corn roots there was some indication of concentration but no regularity. Possibly the growth substances from the two sources were not identical, but the effect on the Lemna was the same.

The prepared extracts increased the rate of reproduction of Lemna but in both cases inhibited the growth of roots—an inhibitory effect reported by Thimann (6) for oat seedlings in auxin solutions. The stimulation of Lemna by the auxins was accompanied by a decrease in both chlorophyll and size of fronds. These effects were similar to those of the beef liver extract.

INFLUENCE OF SYNTHETIC GROWTH-PROMOTING SUBSTANCES ON REPRODUCTION OF LEMNA

Beta-indolylacetic, phenylacetic and phenylpropionic acids, organic compounds known to stimulate the growth of green plants, were added to sterile inorganic media in concentrations varying from 0.0001 to 100 mg. per liter of media. Water solutions of the compounds were sterilized by filtration through Pasteur Chamberland filter candles and combined aseptically with the sterile inorganic media.

Beta-indolylacetic acid (identical with heteroauxin, one of the auxins produced in the coleoptile tips of *Avena*), in any of the concentrations used did not stimulate the rate of reproduction of the Lemna; 0.1 and 1.0 mg. per liter of media caused a marked inhibition of root growth and a decrease in both chlorophyll and size of fronds, and 10 mg. or more killed the plants.

The lower concentrations (0.0001 mg. up) of phenylacetic and phenylpropionic acids produced a very slight increase in the rate of reproduction of Lemna, while 0.1 and 1.0 mg. per liter of media decreased that function and inhibited root growth, but increased frond size. Ten mg. per liter of phenylacetic acid and amounts somewhat higher than 10 mg. for phenylpropionic acid, were toxic to the plants.

No permanent changes were effected in Lemna by concentrations of the synthetic compounds lower than the amounts which killed the plants. The treated Lemna, when transferred to inorganic media, returned to normal health and growth within three weeks.

The results obtained from the stimulation of Lemna, both by the growth-promoting extracts and by the pure synthetic growth-promoters, indicated that any marked effects produced were at the expense of some other part or function of the plant. There is thus some confirmation of Leonian's (4) view that the synthetic auxins may be frequently growth-inhibiting rather than growth-inducing.

SYNTHESIS OF VITAMIN B₁ BY LEMNA GROWN UNDER VARIED CONDITIONS

The presence of vitamin B₁ in green plants and its relation to plant growth-promoting substances (Bonner, 1) suggested that healthy and normal Lemna should synthesize the vitamin regardless of conditions of growth.

Lemna were grown under the environmental conditions described for "Pantothenic Acid," and also in a soil-water mixture in sunlight in the presence of microorganisms and organic matter. Excess plants from these cultures were air-dried and included in the diet of vitamin B₁-depleted rats as the only source of that vitamin. Under the growth conditions used, the results from two rat feeding experiments showed that vitamin B₁ was synthesized by Lemna and that a greater quantity was produced by sterile plants grown in inorganic media under electric light than by non-sterile plants grown in a soil-water mixture in sunlight.

SUMMARY

1. An extract of beef liver (pantothenic acid) and extracts of roots and shoots of germinated corn (auxins) increased the rate of reproduction of Lemna. The stimulation was accompanied by an inhibition of root growth and a decrease in both chlorophyll and size of the fronds.

2. Beta-indolylacetic acid (synthetic heteroauxin) in concentrations from 0.0001 to 100 mg. per liter of media did not stimulate the reproduction of the plants; 0.1 and 1.0 mg. inhibited root growth and decreased both chlorophyll and size of fronds, and 10 mg. or more killed the plants.

3. Phenylacetic and phenylpropionic acids in concentrations of 0.0001 to 0.01 mg. per liter of media gave a very slight stimulation to the rate of reproduction; 0.1 and 1.0 mg. per liter decreased that function, inhibited root growth and caused an increase in size of fronds. Ten mg. or more of phenylacetic acid and amounts somewhat higher than ten mg. of phenylpropionic acid were toxic.

4. Lemna, irrespective of the presence or absence of microorganisms and organic matter, were shown to synthesize vitamin B₁. A greater quantity was produced by sterile plants in inorganic media than by non-sterile Lemna in a soil-water mixture.

LITERATURE CITED

1. BONNER, J.
1937. Role of vitamins in plant development. *Bot. Rev.* 3:616-640.
2. CLARK, N. A.
1932. Technique for the growth of Lemna under sterile conditions with controlled temperature and light. *Iowa State Coll. Jour. Sci.* 7:13-16.
3. CLARK, N. A., and E. M. ROLLER
1931. The stimulation of Lemna major by organic matter under sterile and non-sterile conditions. *Soil Sci.* 31:299-309.
4. LEONIAN L. H., and V. G. LILLY
1937. Is heteroauxin a growth promoting substance? *Amer. Jour. Bot.* 24:135-139.

5. MCBURNEY, C. H., W. B. BOLLEN and R. J. WILLIAMS
1936. Pantothenic acid and the nodule bacteria-legume symbiosis. *Proc. Natl. Acad. Sci.* 21:301-304.
6. THIMANN, K. V.
1937. On the nature of inhibitions caused by auxin. *Amer. Jour. Bot.* 24:407-412.
7. WENT, F. W.
1935. Auxin, the plant growth hormone. *Bot. Rev.* 1:162-182.
8. WILLIAMS, R. J., C. LYMAN, G. GOODYEAR, J. TRUESDAIL and D. HALADAY
1933. "Pantothenic Acid." A growth determinant of universal biological occurrence. *J. Amer. Chem. Soc.* 55:2912-2927.

STRESSES IN MODERATELY THICK RECTANGULAR PLATES¹

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In 1922 G. D. Birkhoff (1) suggested a method for solving plate problems which involves the representation of the displacements by power series. C. A. Garabedian (2) and H. W. Sibert (3) used this idea in developing methods for solving problems in moderately thick circular plates. Garabedian (4) has also published some results for uniformly loaded rectangular plates.

The author gives a solution for the displacements in an elastic isotropic moderately thick rectangular plate under the action of any given load which can be expressed as a polynomial in x, y continuous over the entire plate and with prescribed boundary conditions at the edges. The method, similar to that used by Sibert (3) for circular plates, is based on the assumption that the components of displacement can be developed in positive integral powers of z . In this type of problem, the displacements must satisfy (a) the stress equations of equilibrium throughout the plate, (b) the surface traction conditions on the upper and lower faces, and (c) the boundary conditions at the edges.

GENERAL THEORY

The displacements, u , v , w , are given by

$$(1) \quad u = \sum_{n=0}^{\infty} U_n \frac{z^n}{n!}, \quad v = \sum_{n=0}^{\infty} V_n \frac{z^n}{n!}, \quad w = \sum_{n=0}^{\infty} W_n \frac{z^n}{n!},$$

where U_n , V_n , and W_n are continuous and continuously differentiable functions of x, y . When these displacements are substituted in the equations of equilibrium (A. E. H. Love (5), p. 134) recurrence relations are obtained by which U_n , V_n , and W_n are expressed in terms of U_0 , V_0 , W_0 , U_1 , V_1 , and W_1 .

A right-handed coordinate system with the faces of the plate $z = \pm h$, $x = 0$, $x = a$, $y = 0$, and $y = b$ is used. When the displacements are made to satisfy the surface traction conditions (Love (5), p. 77 and p. 101) two simultaneous systems of partial differential equations are obtained. One system involves only W_0 , U_1 , and V_1 ; the other, only U_0 , V_0 , and W_1 .

For simplicity the problem is restricted to the case of a normal surface load only. The solution for the case of a shearing load is very similar to this case. By superposing these solutions, the results for more complicated problems can be obtained.

The general solutions of the two simultaneous systems of equations are obtained by an indirect process due to Sibert (3).

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$$\text{Let } \Delta_2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2}; \quad D = \frac{4\mu h^3}{3(1-\sigma)} = \frac{2Eh^3}{3(1-\sigma^2)}; \quad -p_1 \text{ and } -p_2,$$

where p_1 and p_2 are functions of x, y , equal the difference and sum, respectively, of the normal loads on the top and bottom of the plate. The general solutions are

$$(2) \quad \Delta_4 W_0 = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n} p_1}{D} [(2-\sigma)d_n - b_n],$$

$$(3) \quad \Delta_4 U_1 = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n} \left(\frac{\partial p_1}{\partial x} \right)}{D} [\sigma d_n + b_n],$$

$$(4) \quad \Delta_4 V_1 = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n} \left(\frac{\partial p_1}{\partial y} \right)}{D} [\sigma d_n + b_n],$$

$$(5) \quad \Delta_2 \left(\frac{\partial U_1}{\partial x} + \frac{\partial V_1}{\partial y} \right) = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n} p_1}{D} [\sigma d_n + b_n],$$

$$(6) \quad \Delta_2 W_1 = - \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n+2} p_2}{4\mu} [(1-\sigma)c_n + \sigma a_n],$$

$$(7) \quad \Delta_4 U_0 = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n+2} \left(\frac{\partial p_2}{\partial x} \right)}{4\mu} [\sigma c_n + (1-\sigma)a_n],$$

$$(8) \quad \Delta_4 V_0 = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n+2} \left(\frac{\partial p_2}{\partial y} \right)}{4\mu} [\sigma c_n + (1-\sigma)a_n],$$

$$(9) \quad \Delta_2 \left(\frac{\partial U_0}{\partial x} + \frac{\partial V_0}{\partial y} \right) = \sum_{n=0}^{\infty} \frac{h^{2n} \Delta_{2n+2} p_2}{4\mu} [\sigma c_n + (1-\sigma)a_n],$$

where $a_0 = 0$, $b_0 = 1$, $c_0 = 1$, $d_0 = 0$,

$$a_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+2)a_{n-1-i} - (i+1)c_{n-1-i}}{(2i+3)!} \quad (n = 1, 2, 3, \dots),$$

$$b_n = 6 \sum_{i=0}^{n-1} (-1)^i \frac{(i+2)[(i+2)b_{n-1-i} - (i+1)(1-\sigma)d_{n-1-i}]}{(2i+5)!} \quad (n = 1, 2, 3, \dots),$$

$$c_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+1) a_{n-1-i} - i c_{n-1-i}}{(2i+2)!} \quad (n = 1, 2, 3, \dots),$$

$$d_n = \sum_{i=0}^{n-1} (-1)^i \frac{(i+1) b_{n-1-i} - i(1-\sigma) d_{n-1-i}}{(1-\sigma)(2i+2)!} \quad (n = 1, 2, 3, \dots).$$

The displacements u, v, w are readily obtained when the six functions U_0, V_0, W_0, U_1, V_1 and W_1 are known. Therefore, one can say that the differential equations (2) to (9) inclusive define the displacements. Furthermore, the displacements defined by these differential equations satisfy the equilibrium equations and the surface traction conditions for any normal load which can be expressed as a polynomial in x, y continuous over the entire plate. It remains to solve these differential equations subject to particular sets of edge conditions.

$$\text{NORMAL LOAD, } p_1(x, y) = p_2(x, y) = P \left(\lambda + \frac{\alpha x}{a} + \frac{\beta y}{b} \right)$$

$$\text{The displacements are found for a normal load } P \left(\lambda + \frac{\alpha x}{a} + \frac{\beta y}{b} \right)$$

on the top surface of the plate where a and b are the horizontal dimensions of the plate, λ, α , and β are arbitrary constants, and P is a uniform load per unit of area. In each case the edges $x = 0, a$ are pinned. The three cases where the edges $y = 0, b$ are pinned, clamped, and free are solved. In each case the moderately thick plate solution consists of the corresponding thin plate solution plus corrections. Each moderately thick plate solution reduces to that given by Garabedian (4) for the case $\lambda = 1$ and $\alpha = \beta = 0$.

LITERATURE CITED

1. BIRKHOFF, G. D.
1922. Circular plates of variable thickness. *Phil. Mag. Series 6*, 43:953-962.
2. GARABEDIAN, C. A.
1923. Circular plates of constant or variable thickness. *Trans. Am. Math. Soc.*, 25:343-398.
3. SIBERT, H. W.
1931. Moderately thick circular plates with plane faces. *Trans. Am. Math. Soc.*, 33:329-369.
4. GARABEDIAN, C. A.
Compt. rend. Acad. sci. Paris, 178(1924), 180(1925), 181(1925), 186(1928), 195(1932.)
5. LOVE, A. E. H.
1927. *A treatise on the mathematical theory of elasticity*. 4th ed. University Press, Cambridge.

SULFUR ANALOGS OF FURAN TYPES¹

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The chemistry of furan analogs has been extended in an investigation of the sulfur analog, dibenzothiophene. A review of the natural occurrence and methods of preparation of dibenzothiophene, and of several general methods for the ring closure synthesis of dibenzothiophene derivatives has been made.

The most extensive study of the orientation in dibenzothiophene is that of Courtot and co-workers. They have shown that halogenation, nitration, and sulfonation affect the 2- and 2,8-positions (*para* to the sulfur linkage). In extending this study it has now been found that acetylation by the Friedel-Crafts reaction, using acetyl chloride and anhydrous aluminum chloride in carbon disulfide gives 2-acetyldibenzothiophene, m.p. 111°. The structure of this ketone was established by oxidation with iodine and sodium hydroxide to dibenzothiophene-2-carboxylic acid, first prepared by Courtot, Nicolas and Liang². Methyl dibenzothiophene-2-carboxylate, m.p. 74-75°.

Dibenzothiophene has been found to undergo metalation with various organolithium compounds to give, after carbonation with solid carbon dioxide, dibenzothiophene-4-carboxylic acid, m.p. 252-253°; methyl ester, m.p. 95°. The structure of this acid was established by treating 4-dibenzothiényllithium with dimethyl sulfate to give 4-methyldibenzothiophene, m.p. 66.5°, and comparing it with an authentic specimen prepared by the reaction of 3-methyl-2,2'-dihydroxybiphenyl and phosphorus pentasulfide. The yields of dibenzothiophene-4-carboxylic acid from *n*-butyl-, phenyl-, α -naphthyl-, and *p*-anisyllithium were found to be approximately 65, 12, 7.6 and 0 per cent, respectively. An anomalous behavior of *p*-anisyllithium is noted which may account for the absence of any metalation.

Phenylcalcium iodide metalates dibenzofuran in the same position as do the organolithium compounds. However, treatment of dibenzothiophene with phenylcalcium iodide, followed by carbonation, gave a monobasic acid different from the 2- and 4-acids; m.p. 300-305° (dec.); methyl ester, m.p. 129-130°. Decarboxylation of the acid gave dibenzothiophene. This acid is probably dibenzothiophene-3-carboxylic acid.

Mercuration of dibenzothiophene by fusion with mercuric acetate gives what appears to be a monoacetoxymercuri derivative [m.p. 215° (dec.)] which is contaminated with a small amount of polymercurated material. The position of mercuration is unestablished.

Treatment of 4-dibenzothiényllithium with oxygen gives 4-hydroxydibenzothiophene, m.p. 167°. This phenol gives a green color with ferric chloride and, upon nitration with concentrated nitric acid in warm acetic acid solution, a dinitro derivative, dark red crystals, m.p. 204° (dec.).

¹ Original thesis submitted March, 1938. Doctoral thesis number 459

² Courtot, Nicolas and Liang, *Compt. rend.*, 186, 1624 (1928).

Methylation of 4-hydroxydibenzothiophene gives 4-methoxydibenzothiophene, m.p. 123°.

When 4-hydroxydibenzothiophene is treated with sodium bisulfite and concentrated aqueous ammonia, 4-aminodibenzothiophene is obtained, m.p. 110°; acetyl derivative, m.p. 198°. A better synthesis of the 4-aminodibenzothiophene consists of treating crude 4-bromodibenzothiophene with concentrated aqueous ammonia and cuprous bromide in a bomb. The 4-bromodibenzothiophene is obtained by the reaction of bromine with 4-dibenzothiophenyllithium and need not be separated from the accompanying dibenzothiophene. Bromination of 4-acetaminodibenzothiophene gives a monobromo derivative, m.p. 254°, which is probably 1-bromo-4-acetaminodibenzothiophene.

With the original intention of preparing alkylated imidazoles for physiological tests, the nitration of 2-acetaminodibenzothiophene was studied. 2-Acetaminodibenzothiophene was first reported³ as melting at 168° but was found to melt at 178°. Because of the difficulty of obtaining 2-nitrodibenzothiophene in good yield by direct nitration, for conversion into the desired amine, two new routes to the acetamino derivative were developed. The first involves the bromination of dibenzothiophene, amination of the bromo derivative with ammonia, and acetylation of the resulting amine. The other method consists of effecting the Beckmann rearrangement of the oxime (m.p. 160-161°) of 2-acetyldibenzothiophene. By either route, the overall yield of acetamino compound, based on the dibenzothiophene, is at least 38 per cent. Nitration of 2-acetaminodibenzothiophene with fuming nitric acid in acetic anhydride gives a nitro-acetamino compound, m.p. 209°, in 67 per cent yield, and a small amount of a nitro-aminodibenzothiophene is also formed, m.p. 248-250° (dec.). An attempt to hydrolyze nitro-2-acetaminodibenzothiophene with alcoholic hydrochloric acid gave an as yet unidentified nitrogen-free material, m.p. 88°.

Dibenzothiophene is best prepared by heating biphenyl with sulfur in the presence of aluminum chloride. The possibility of obtaining substituted dibenzothiophenes by starting with substituted biphenyls was investigated but the polymerizing effect of the catalyst under conditions necessary to cause an evolution of hydrogen sulfide seems quite marked, and from *p*-bromobiphenyl the only product which could be isolated was dibenzothiophene, in 15 per cent yield. The use of a milder condensing agent is apparently not practicable but there are some indications which point to the success of the method when chlorobiphenyls and a lower temperature are employed.

Two polycyclic, condensed-ring compounds containing the dibenzothiophene system have been synthesized for physiological tests. The first, 1-keto-1,2,3,4-tetrahydrothiobrazan (β - or γ -) was prepared as follows. Succinic anhydride was condensed with dibenzothiophene in the presence of anhydrous aluminum chloride to give β -2-dibenzothienoylpropionic acid, m.p. 160.5-161°. This was reduced with zinc and hydrochloric acid to give γ -2-dibenzothiophenylbutyric acid, m.p. 131°. Cyclodehydration of the latter with sulfuric acid gave the 1-keto-1,2,3,4-tetrahydrothiobrazan, m.p. 178°.

The second condensed-ring compound was prepared in a similar manner from dibenzothiophene and phthalic anhydride. The resulting *o*-2-

³ Courtot and Pomonis, *Compt. rend.*, 182, 931 (1926).

dibenzothienoylbenzoic acid (unstable hydrate, m.p. 120-125°; ethyl ester, m.p. 105-106°) was cyclized by fusion with a mixture of aluminum chloride and sodium chloride to give yellow crystals of a thionaphthoanthraquinone, m.p. 285-286°.

When dibenzothiophene is treated with sodium in liquid ammonia a dihydro derivative is obtained, m.p. 76°; picrate, red needles, m.p. 105°. By analogy with naphthalene, phenanthrene, and dibenzofuran, this is probably 1,4-dihydrodibenzothiophene. When treated with bromine in chloroform solution at 0°, the dihydrodibenzothiophene absorbs a mole of bromine without evolution of hydrogen bromide. The resulting oil loses hydrogen bromide when warmed and gives an almost quantitative yield of dibenzothiophene. This behavior of the bromine addition product is analogous to that of the bromide of 1,4-dihydronaphthalene⁴.

When 1,4-dihydrodibenzothiophene is treated with phenyllithium under conditions identical with those employed in metalations, dibenzothiophene is obtained in almost quantitative yield, along with an appreciable amount of benzene and a white solid which behaves like lithium hydride. It was subsequently shown that phenyllithium is cleaved by hydrogen in the absence of a catalyst to give benzene and lithium hydride. The readily obtainable phenylisopropylpotassium exerts a similar dehydrogenating effect on the dihydrodibenzothiophene.

Earlier work on the reduction of organometallic compounds has been done in this country by Adkins⁵. In 1932 he established a series of organometallic compounds in the order of their decreasing ease of reduction as follows, R_2Mg , R_2Zn , R_4Pb . The compound RLi (phenyllithium) was found to apparently undergo reduction with greater ease than reported for the magnesium compound, thus bringing it into this series before the others. The series, it will be noticed, is then in the order of decreasing reactivity as established by other methods.

⁴ Bamberger and Lodter, *Ber.*, 20, 1706 (1887).

⁵ Zartmann and Adkins, *J. Am. Chem. Soc.*, 54, 3398 (1932).

TOXICITY OF METHYL FORMATE, ETHYLENE OXIDE AND METHYL BROMIDE, IN ADMIXTURE WITH CARBON DIOXIDE, TO THE RUST-RED FLOUR BEETLE¹

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The literature relating to the use of CO₂ in admixture with fumigants shows not only that moderate concentrations of this gas may have a marked effect on the respiratory system of insects, but also that the quantity which is required to give the maximum insecticidal effect will vary with the kind of fumigant with which it is combined. The writer gives data on the proper proportions in which the CO₂ should be combined with methyl formate, ethylene oxide and methyl bromide, and shows that the stimulative effect produced by the CO₂ may be partly or wholly nullified if the percentage in the gaseous mixture is increased beyond the amount which is found to give the maximum increase in toxicity.

An analysis of the proprietary fumigant mixtures containing CO₂ in admixture with methyl formate, ethylene oxide and methyl bromide indicates that the percentage of CO₂ incorporated into these mixtures is based upon the quantity which is required to remove the fire-hazard of the fumigant, rather than upon the concentration which would affect the maximum increase in the toxicity of the fumigant. A better approach to the problem of determining the most effective fumigant-CO₂ mixture would be to ascertain the actual toxicological effects attending the exposure of insects to these fumigants when they are admixed with CO₂ in various proportions. The writer has attempted to do this in the present investigation.

The apparatus employed in the investigation was developed by the writer and has the advantage of permitting the vapors of volatile fumigants, instead of the liquid substance, to be introduced into partially evacuated 5-liter balloon flasks. The amounts are measured by means of a mercury manometer and the concentrations are expressed as milligrams per liter or per cent by volume.

Tests were first conducted to determine the concentrations of methyl formate, ethylene oxide and methyl bromide which are required to kill 100 per cent of the adults of the rust-red flour beetle (*Tribolium castaneum*) in 5 hours at a temperature of 27° C. The results are as follows: (1) methyl formate, 25.0 mg. per liter, (2) ethylene oxide, 17.5 mg. per liter and (3) methyl bromide, 8.75 mg. per liter.

The addition of CO₂ has a marked effect on the toxicity of methyl formate to the adults of the above insect. When 1 per cent of CO₂ is added to atmospheres containing 25.0 mg. of methyl formate per liter, the period of exposure required to kill all of the beetles is decreased to 3.5 hours, as compared with 5 hours when no CO₂ is present in the gaseous mixture. The addition of 5, 10, 20 and 40 per cent of CO₂ reduces the time

¹ Original thesis submitted December, 1937. Doctoral thesis number 454.

required to kill all of the insects to 2.5, 2, 1.5 and .75 hours, respectively. Concentrations of CO_2 in excess of 40 per cent apparently impart no additional increase in toxicity to methyl formate.

The results obtained with ethylene oxide (17.5 mg. per liter) indicate that the addition of 1 per cent of CO_2 does not increase the toxicity of this chemical to *T. castaneum*. When 5 per cent of CO_2 is added to ethylene oxide, however, the length of time necessary to affect a 100 per cent kill of the beetles is reduced to 3 hours, as compared with 5 hours when no CO_2 is present. A further reduction in time to 1.5 hours is observed when the concentration of CO_2 is increased to 10 per cent. All of the insects are killed in three-fourths of an hour when CO_2 is admixed at the rate of 20, 40, 60, 80 and 99.8 per cent. The addition of 60 per cent of CO_2 apparently produces the maximum insecticidal effect with ethylene oxide. Mixtures containing 80 and 99.8 per cent of CO_2 appear to be somewhat less toxic than those which have 60 per cent incorporated into the gaseous mixture.

The results also show that the addition of CO_2 does not increase the toxicity of methyl bromide to the same extent as observed with methyl formate and ethylene oxide. Atmospheres of methyl bromide (8.75 mg. per liter) containing 1 per cent and 99.8 per cent of CO_2 have about the same degree of toxicity to *T. castaneum* and are only slightly more toxic than methyl bromide without CO_2 . Both mixtures require 4.5 hours to kill all of the insects, as compared with an exposure period of 5 hours without the CO_2 . Likewise, methyl bromide plus 80 per cent of CO_2 is apparently no more toxic than mixtures having 5 per cent of CO_2 , as an exposure of 4 hours is required to kill 100 per cent of the beetles with each concentration. Ten to 40 per cent of CO_2 produce the maximum stimulative effect when used in admixture with 8.75 mg. of methyl bromide per liter, and the results indicate that mixtures containing 20 per cent are the most effective, especially in the lower range of mortalities.

The data presented in the thesis indicate, therefore, that the maximum insecticidal effect of methyl formate, ethylene oxide and methyl bromide is obtained with concentrations of 40, 60 and 20 per cent of CO_2 , respectively, and that the addition of CO_2 in excess of these percentages may either impart no further increase in toxicity to the mixture, or may actually decrease the effectiveness of the gaseous mixture.

THE CRAWFISHES OF MISSISSIPPI, WITH SPECIAL REFERENCE TO THE BIOLOGY AND CONTROL OF DESTRUCTIVE SPECIES¹

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This study of Mississippi crawfishes² resulted when a group of planters in the Northeast Prairie soil belt of Mississippi in 1932 requested the Mississippi Agricultural Experiment Station to develop control measures for a species which was very destructive to farm crops in that section. While conducting that investigation the writer became interested in learning how many species occurred in Mississippi. Collections were made in many parts of the state. The collection of the Zoology and Entomology Department of Mississippi State College, largely unidentified until the inception of this study, has been of much assistance. Comparisons of specimens with types in the Harvard Museum of Comparative Zoology and in the National Museum aided greatly with the identifications.

HISTORICAL

The earliest of any taxonomic work on crawfishes from Mississippi was by Charles Girard, who named the first species in 1852. In 1870 Hagen listed three species from the state in his "Monograph of the Astacidae." Faxon reported seven in 1885 and ten in 1914. The catalog of the National Museum now lists twelve authentic species. This study has increased the total number of known Mississippi species to 22, of which eight are new species and subspecies. Types are being retained at present in the collection of the Zoology and Entomology Department of Mississippi State College but will ultimately be deposited in the National Museum and the Harvard Museum of Comparative Zoology.

ECONOMIC IMPORTANCE

The only crawfishes in the United States greatly destructive to farm crops are *Cambarus hagenianus* Faxon and its subspecies, which apparently occur only in Mississippi and Alabama. Other species may drain ponds as a result of their burrows in dams, or may cause rivers at flood stage to break through levees weakened by their tunnels.

Of minor importance for human food, crawfish are very valuable in the diet of many animals such as raccoons, minks, opossums, bears and alligators. Wading birds, as well as some ducks, consume large numbers. Trout, bass, crappie, perch and other fish make them a staple item in their diets.

¹ Original thesis submitted July, 1937. Doctoral thesis number 439.

² The term "crawfish" is used in this paper since it is the common name for these animals throughout the South and in other rural sections of the United States. It was early given approval in a scientific publication on Crustacea by Thomas Say in 1817.

GENERAL ANATOMY AND BIOLOGY

These subjects are treated in so many zoological texts it is unnecessary to discuss them here.³

CLASSIFICATION

Subgenera of the Genus *Cambarus* Erichson

All crawfishes in the United States east of the Rocky Mountains belong to the genus *Cambarus* Erichson. Mississippi apparently has representatives of all the subgenera known in the United States at this time. The following are recorded with the Mississippi species of each.⁴

Subgenus *Girardiella*, new subgenus (Faxon's Group II)

1. *Cambarus hagenianus* Faxon
2. *Cambarus hagenianus evansi*, new subspecies
3. *Cambarus hagenianus carri*, new subspecies
4. *Cambarus hagenianus forestae*, new subspecies

Subgenus *Ortmannicus* Fowler (Faxon's Group III)

5. *Cambarus blandingii acutus* Girard
6. *Cambarus hayi* Faxon
7. *Cambarus clarkii* Girard
8. *Cambarus cookae*, new species
9. *Cambarus evictus*, new species
10. *Cambarus spiculifer* LeConte
11. *Cambarus versutus* Hagen

Subgenus *Paracambarus* Ortmann (Faxon's Group IV)

12. *Cambarus harnedi*, new species

Subgenus *Cambarellus* Ortmann (Faxon's Group V)

13. *Cambarus shufeldtii* Faxon

Subgenus *Faxonius* Ortmann (Faxon's Group VI)

14. *Cambarus lancifer* Hagen
15. *Cambarus mississippiensis* Faxon
16. *Cambarus creaseri*, new species

Subgenus *Cambarus* Fowler (Faxon's Group VII)

17. *Cambarus diogenes* Girard
18. *Cambarus diogenes ludovicianus* Faxon
19. *Cambarus latimanus* LeConte
20. *Cambarus argillicola* Faxon
21. *Cambarus lobdelli*, new species

Subgenus *Faxonella* Creaser

22. *Cambarus clypeatus* Hay

³The thesis contains a chapter on these subjects with an illustration of all the parts of a crawfish useful in identification.

⁴A key to the crawfishes of Mississippi, descriptions of new species, and distinguishing characters of others in the state, are included in the thesis.

THE BIOLOGY AND CONTROL OF CAMBARUS HAGENIANUS FAXON AND ITS SUBSPECIES

BIOLOGY

These crawfishes inhabit the lime soils of Mississippi and Alabama. Each subspecies is separated from the others but their habits are very similar. To secure information on their habits, flashlight observations on many rainy nights and the excavation of many burrows were supplemented by laboratory studies of individuals in glass cages of soil standing in water. They live in deep burrows and never visit ponds or streams as do most other species. Their diet is chiefly vegetarian and on rainy spring nights they often destroy entire fields of young corn and cotton. They can live long periods without food, eight or nine months in some cases. They survive temperatures of 38° F. and probably lower but die quickly at 100-102° F. Only a small percentage of the females produce young each year. Oviposition occurs in the fall, the young usually hatching from October to December and remaining in the burrow with the mother until the winter or spring of the second year, or 12 to 18 months. The average number of young is about five or six, the maximum found in any nest being 20. Apparently they become sexually functional in about three years and attain full size when six or seven years old.

CONTROL

The best control is the application of poison to the burrows when the water level is only a few inches from the surface of the ground, or from January to May in most years. Pyrethrum, commercial creosote stock dips, coal tar creosote, orthodichlorobenzene, sodium cyanide, turpentine, ortho cresole, cresylic acid, miscible pine oil, nicotine, carbon bisulphide, phenothiazine and calcium cyanamid were toxic in laboratory tests in the order named. Ineffective materials included the arsenicals, rotenone, picric acid, pyridine, resorcinol, thallium sulphate and several others. Pyrethrum was ineffective at the temperatures in the burrows.

The creosote stock dips are recommended on account of their low cost and ease of application. With infestations of 10,000 burrows per acre the cost of material is about 50 to 60 cents per acre. Where the stock dips are not available at low cost, home-made emulsions of turpentine and creosote are advised.

Applications on small areas may be made with compressed air sprayers. On large plantations a double-acting force pump, mounted on a wagon and supplying several leads of hose, is very satisfactory. Splendid results have been secured on large-scale tests covering several hundred acres.

SOME FACTORS AFFECTING THE RAMAN FREQUENCIES OF THE CARBON-MERCURY BOND¹

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Theoretical considerations indicate that Raman spectra studies should show how the carbon-mercury bond varies as the radical attached to the mercury is changed.

Hixon and Johns² found that the polar properties of a series of compounds such as RNH_2 or RCOOH which contain one polar group could be expressed as a function of the electron sharing ability of the radical R. Later studies on a number of equilibria supported the original hypothesis and confirmed the order in which the radicals were arranged. Studies of refractivity and thermal stability of compounds of the type R_2Hg and RHgCN by Carr³ have added some purely physical measurements to the physico-chemical studies.

All these experiments measured the electron sharing ability indirectly. The present investigation was undertaken as a direct study of the bond between the radical and another group or atom. A series of R_2Hg compounds was chosen for the study because refractivity and thermal stability data were available for a number of these compounds.

The apparatus consisted of a high speed spectograph and an auxiliary excitation unit for producing the Raman spectra. The excitation unit was in the form of an elliptical cylinder and was made from polished sheet aluminum. The tube holding the liquid under investigation was placed in a vertical position inside a condenser at one focus of the ellipse. The direct current mercury arc used as a light source was placed at the other focus of the ellipse. A total reflecting prism located directly below the Raman tube served to send light through a condensing lens into the spectograph. A slit in the elliptical reflector midway between the arc and the Raman tube allowed a glass light filter to be inserted. Solutions containing dissolved salts were circulated through the condenser to act as additional light filters and to keep the solution or liquid in the Raman tube at the proper temperature.

The following compounds' spectra were photographed: Diethylmercury, diethylmercury in carbon tetrachloride, diethylmercury in acetone, di-n-propylmercury, di-n-propylmercury in carbon tetrachloride, dibenzylmercury in carbon tetrachloride, dibenzylmercury in acetone, diphenylmercury in carbon tetrachloride, mercuric cyanide in acetone and mercuric chloride in acetone. Attempts were made to obtain the spectra of di-p-tolylmercury and di-alpha-naphthylmercury. Due to the very low solubility of these compounds no results were obtained for them.

¹ Original thesis submitted June, 1938. Doctoral thesis number 476.

² Hixon and Johns, *J. Am. Chem. Soc.* 49, 1786 (1925).

³ Carr, Unpublished work, this laboratory. (1934.)

The frequencies observed for the compounds investigated are listed in table 1.

TABLE 1. *Frequencies of a series of organomercury compounds, R_nHg*

Propyl	Ethyl	Benzyl	Phenyl	Chloride	Cyanide
171 (0)	140 (1)	175 (m)	152 (5)	321 (5)	276 (w)
276 (3)	212 (2)	260	340		
381 (3)	264 (3)	325	382		
501 (10)	329 (0)	560 (5)	555 (s)		
585 (4)	486 (8)	638	580		
791 (0)	562 (0)	690	619		
863 (1)	633 (0)	810	652 (s)		
1016 (2)	958 (1)	996 (s)	700		
1084 (2)	1008 (3)	1070	997 (s)		
1158 (10)	1055 (2)	1153	1060		
1267 (00)	1178 (6)	1205	1185		
1326 (00)	1370 (1)		1262		
1446 (2)	1421 (3)		1370		
	1455 (3)				

No separation of the frequencies listed in table 1 has been made on the basis of the solvent used. Variations due to the solvent were less than the experimental error in measuring the frequencies.

The molecules were considered as linear triatomic systems for the purpose of calculating force constants for the carbon-mercury bond. Table 2 shows the frequencies selected as the fundamentals and the force constants for the carbon-mercury bond. These constants are considered as representing the restoring forces which act on the group which vibrates.

TABLE 2. *Fundamental frequencies and force constants for R_nHg compounds*

Radical	ν_1	ν_2	ν_3	k_1	k_a
Propyl	501	171	585	5.4	0.27
Ethyl	486	140	562	4.7	0.13
Methyl ^a	515	156	565	3.2	0.09
Benzyl	560	175	638	5.5	0.63
Phenyl	555	152	652	6.9	0.38
Chloride	321			2.2	
Cyanide	276			1.2	

In table 2, k_1 represents the stretching constant and k_a represents the bending constant. These and other constants calculated indicate that the radicals vibrate almost as units in the aliphatic compounds. The radicals do not vibrate as units in the aromatic compounds.

The values obtained for the bending constants indicate that the aliphatic compounds are much more flexible than the aromatic compounds.

^aThompson and Linnett, *Proc. Roy. Soc. (London)* A 160, 139 (1937).

The force constants and other information obtained from the Raman spectra were compared with the information given by thermal stability and refraction studies on the R_2Hg compounds.

If the radicals are placed in the order indicated by the dissociation constants of the amines the force constant curve shows a maximum. This maximum is at the same point as the minimum thermal stability and maximum refractivity of mercury in the same compounds.

CHARACTERIZATION OF CERTAIN PRODUCTS OF STARCH- ENZYME DIGESTION¹

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Three fairly definite materials appear to be the result of beta amylase action on starches. They are: (1) The material which flocculates during the digestion, (2) 60-67 per cent maltose, (3) "residual dextrins" or "alpha amyloextrin." The purpose of this investigation was to prepare and study the flocculent material and residual material from beta amylase action on a series of starches.

The course of enzyme action was studied in order to determine the length of time to allow the enzyme to act and to determine any differences in beta amylase action on starches of different origin. The effects of the enzyme on viscosity, "residual starch"², and reducing action of the substrates were measured. However, the results of these determinations on the same digestion could not be correlated.

In the digestion of two per cent soluble potato starch with oat enzyme the viscosity had reached a minimum after 30 minutes of digestion, but the amount of material precipitated by 55 per cent alcohol was still decreasing after one hour. These two methods measure different effects of amylase action. Neither method is strictly a measure of the decrease in molecular size of the substrate as digestion proceeds. The reducing action against alkaline copper was still increasing after 50 minutes digestion. Similar experiments on the digestion of natural corn and potato starches with wheat amylase gave no correlation of the different methods. The results indicated that determinations of viscosity, "residual starch," and reducing action by Fehling's gravimetric method were not applicable to following the hydrolysis of natural starches.

Two other methods of following the reducing action during enzyme digestion were worked out. The modified Hagedorn and Jensen³ sugar method gave satisfactory results during digestion of starches that had been boiled. However, when the substrates had been heated below the boiling point the results with this method were in error due to unswollen starch particles which held the iodine. A potentiometric sugar method was devised to remove this difficulty. The method of Hassid⁴ was modified to a macro determination and the titration of ferrocyanide with ceric sulfate was followed potentiometrically.

A comparison of the rates of hydrolysis of natural and alcoholic HCl treated corn and potato starches was made. The substrates were prepared

¹ Original thesis submitted June, 1938. Doctoral thesis number 472.

² Caldwell and Hildebrand, *J. Biol. Chem.* 111, 411 (1935).

³ Hagedorn and Jensen, *Biochem. Z.* 137, 92 (1923).

⁴ Hassid, *Ind. Eng. Chem. (Anal. Ed.)* 9, 228 (1937).

by boiling, and the beta amylase from soybeans was used⁵. Changes in viscosity and reducing action as measured by the Hagedorn and Jensen method were followed. The decrease in viscosity was very markedly affected by the alcoholic HCl treatment of the starch. Liquefaction of the corn starches proceeded more rapidly than that of the potato starch, either treated or natural. The treatment of the starches had very little effect on the rate of sugar formation by beta amylase. Sugar production proceeded more rapidly during the digestion of corn starch than either natural or treated potato starch. However, after 24 hours the percentage reducing value as maltose was 66-68 per cent in the digestions of the unmodified starches. The value for the digestions of treated corn and potato starches was 61 per cent. It is possible that the treatment of the starches had washed out some more soluble portion of the starch which was digestible by beta amylase.

A study of the effect of temperature of preparing the substrate on the rate of beta amylase action on potato, corn, rice, wheat and tapioca starches was made. Substrates were prepared from these starches by heating 30 minutes at 60°, 70°, 80°, 90°, 100°, and 120° C. The rates of hydrolysis of the starch substrates by soybean amylase were measured by the potentiometric determination of the sugars formed. In every case except rice starch an optimum temperature of preparing the starch for soybean amylase action was indicated—70° for potato, 80° for tapioca, and 90° for corn and wheat starches. Temperatures of 80° or above gave the maximum rate of soybean enzyme hydrolysis. The optimum gelatinization temperatures were much lower for the root starches than for the cereal starches. However, the rates of soybean amylase action on the different substrates prepared at their optimum temperatures were all about the same. The final reducing values were all 60-70 per cent of the original starch. This series contained starches of very high and very low phosphorus and fatty acid content. If these groups are important in enzyme action, some differences should have been noted.

The material which flocculates during enzyme digestion of starches (precipitate A) and the residual material from beta amylase action which is precipitated by 60 per cent alcohol (precipitate B) were prepared from the same series of starches. The precipitates were repeatedly suspended in water and electrodyalyzed in order to remove maltose and ions. The materials were finally dried by grinding under absolute alcohol and ether. When the ether was removed the materials were ground to white powders.

Precipitate A from the cereal starches was formed in larger amounts and settled out of the digestions. Precipitate A from the root starches was transparent when collected in the bowl of the supercentrifuge. Precipitate B from the root starches was a transparent sticky mass before dehydration. Precipitate B from the cereal starches was formed as a curdy white precipitate. One per cent suspensions of the dried powders showed differences also. The suspensions of precipitates A and B from cereal starches were turbid but not viscous. Suspensions of precipitate A from potato and tapioca starches were clear and quite viscous. Precipitate B from the root starches formed clear limpid suspensions.

⁵The characterization of soybean amylase is unpublished work by Mr. J. M. Newton.

These preparations were characterized as to further hydrolysis by fresh beta amylase, reducing action against copper and ferrocyanide, phosphorus and fat content, and recovery in the starch determination of Denny⁶. These data support in the following ways the hypothesis that precipitate A is a portion of precipitate B which for some reason is thrown out of suspension.

1. Precipitates A and B from any one kind of starch are hydrolyzed by beta amylase to about the same degree. Here again the difference between cereal and root starches is noticeable. These materials from root starches are hydrolyzed to a much less degree than precipitates A and B from cereal starches.

2. The reducing values of precipitates A and B are about the same. This is confirmed by both potentiometric determinations and R_{Cu} values.

3. Precipitates A and B from any one kind of starch behave similarly in the starch determination of Denny. Precipitates A and B from root starches do not react at all in this determination.

4. The data on fat and phosphorus content of precipitates A and B indicate that the decreased solubility of precipitate A may be due to either fat or phosphorus. The presence of high content of fatty acids should make the material less soluble, while the phosphorus should make the materials more soluble.

⁶Denny, *J. Assoc. Official Agr. Chem.* 6, 175 (1922).

FACTORS AFFECTING THE OCCURRENCE OF AZOTOBACTER IN IOWA SOILS¹

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A previous investigation of the occurrence and distribution of Azotobacter in Iowa soils showed that many of the more important soil types of the state from the standpoint of total acreage were practically devoid of the bacteria. Barely a third of the 287 samples collected contained the bacteria. Correlation studies of the chemical composition of the samples and the presence of the Azotobacter indicated that the high acidity of the majority of these soils was the most important factor limiting the presence of the organisms and that the available phosphorus content was also of importance in many cases. Other factors being favorable, the results indicated further that the amount of growth which the Azotobacter would make in the soil depended largely upon the organic matter content and upon the pH.

The present investigation was planned to study further and in more detail the results of the correlation studies. Carefully controlled experiments were carried out in which the effect of various soil treatments upon the growth of Azotobacter while in the soil was determined.

The first studies were made of the effect of magnesium ammonium phosphate upon the Azotobacter. It was found that the addition of this compound to the soil plaques or the nitrogen-free agar medium used for the detection of the Azotobacter in soils, brought about a definite increase in the numbers of colonies appearing on the surface of the plates showing that it stimulated the growth of the bacteria. The reason for the stimulative action of the magnesium ammonium phosphate was traced to its content of available nitrogen, although, because of the slight solubility of this compound in water, only small amounts were present in the medium.

In a second experiment, the influence of different fertilizers or combinations of fertilizers upon the growth and activity of Azotobacter in typical samples of Clinton, Grundy and Clarion silt loams was determined. These soils represent a wide variety of soil conditions and, in addition, had been found to be generally lacking in Azotobacter.

The fertilizers used in these experiments were (a) lime to vary the pH of the samples, (b) oat straw to increase the organic matter content, (c) sodium nitrate to determine whether or not small amounts of nitrogen added to these soils would actually stimulate Azotobacter growth and (d) treble superphosphate to increase the amount of readily available phosphate.

The results showed that an addition of lime to these soils was essential for the prolonged growth of the bacteria and that an amount sufficient to raise the pH to near neutrality was all that was necessary to improve

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the environment so that the bacteria would remain active for at least 9 months. The growth of corn, wheat or timothy on the Clinton silt loam or a Clarion fine sand treated with lime did not hinder the growth of the bacteria.

None of the individual treatments other than lime were essential for the growth of the bacteria in these soils. Indeed, except for oat straw, none of the treatments other than lime, regardless of their combination, seemed to exert any influence upon the bacteria. There was some evidence when lime was present, however, that other treatments may aid the growth of the bacteria. This was particularly true for the oat straw treatment in the case of the Clinton silt loam.

The limiting pH value for the growth of the *Azotobacter* in these soils was shown to approach a pH of 6.0 but it was pointed out that the actual limiting value for the different types probably varied with the general soil conditions. Thus, at the same pH, the Grundy silt loam presented a better medium for the growth and activity of the *Azotobacter* than did the Clinton silt loam. The results indicated that the higher organic matter content of the Grundy silt loam may account for this difference.

Having found that a active flora of the *Azotobacter* could be maintained in some acid Iowa soils by the addition of lime sufficient to raise the pH up to about the neutral point, inoculation experiments with the *Azotobacter* were conducted to determine whether or not sufficient nitrogen would be fixed to stimulate the growth of some typical Iowa crops. They were carried out in the greenhouse in triplicate with two soil types which responded to nitrogen fertilization, that is, Clinton silt loam and Clarion fine sand, and three Iowa crops, namely, corn, wheat and timothy.

The inoculation of the Clarion fine sand was completely without effect in altering either the dry weight or total nitrogen content of any of the crops. Tests of this soil for the presence of the *Azotobacter* during the course of the experiment showed that they were present and active when the soil had been treated with lime.

The inoculation of Clinton silt loam with *Azotobacter* was without effect in altering the dry weight and total nitrogen content of the corn and wheat grown upon it. The timothy, however, responded very definitely to the inoculation and a highly significant increase in both the dry weight and total nitrogen content of the crop was obtained. The average increase in the dry weight of the timothy due to the inoculation was about 39.5 per cent; for the total nitrogen content, an increase of about 26.6 per cent occurred. The *Azotobacter* were more active during the course of the experiment in the soils which had been treated with lime. The growth of the bacteria in the unlimed soils, however, was large enough that the response of the timothy to the inoculation did not differ significantly from that on the samples treated with lime.

These results indicate a need for more extended experiments upon the inoculation of soils with *Azotobacter* to test out a greater variety of plants on many different soils. When this is done, definite conclusions regarding the usefulness of the inoculation of soils with *Azotobacter* in our soil management practices may then be reached.

CHARACTERIZATION AND DIFFERENTIATION OF THE "INTER-MEDIATES" OF THE COLON-AEROGENES GROUP OF BACTERIA¹

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An investigation was made of the availability of the nitrogen of nucleic acid and certain of its degradation products for the coli-aerogenes group of bacteria in order to secure an approach, other than the carbohydrate studies, to the systematic and physiological relations of the group. It was hoped that such a study might throw light on the relations of the "intermediate group" to the genera *Escherichia* and *Aerobacter*.

Investigations were concerned primarily with the availability of the nitrogen of nucleic acid and its degradation products. At the same time these compounds were tested in an exploratory manner as to their carbon, or carbon and nitrogen availability, and as to the effect of moderate autoclaving. In addition, the effect of temperature and period of incubation was determined for the pyruvic acid fixation test as suggested by Reynolds (1935).

The compounds tested included: yeast nucleic acid, xanthine, adenine sulphate, uric acid, uracil, allantoin, hydantoin, and urea.

The basal test media consisted of: 0.5 per cent NaCl; 0.02 per cent $MgSO_4$; and 0.004 per cent brom-thymol-blue. To investigate the availability of nitrogen, dextrose, (0.2 per cent) was supplied as the carbon source, and the test compound (0.05 per cent) as the nitrogen source. To investigate the availability of carbon ammonium phosphate (0.2 per cent) was supplied as the nitrogen source, and the test compound (0.05 per cent) as the carbon source. To determine the simultaneous availability of carbon and nitrogen, the test compound (0.5 per cent) alone was employed. Two per cent by volume of phosphate buffer (pH 7.1) was added to all media; if this did not adjust the reaction to approximately pH 7.1, more of the necessary solution was added.

The constituents were dissolved in conductivity water at a temperature below boiling. All media were sterilized by filtration through Chamberlain candles. Inoculation consisted of a one mm. loop of a 24 hour broth culture. Incubation was for five days at 30° C. (temperature of the medium).

Availability of nitrogen was judged by two criteria: (1) acidification of the medium, as it was assumed that the dextrose would be attacked, thus creating an acid reaction, only if there were an available and adequate nitrogen source; and (2) vigor of growth.

The bacteria employed in the nitrogen availability studies included: 106 *Escherichia*; 39 *A. cloacae*; 31 *A. aerogenes*, indol +; 41 *A. aerogenes*, indol —; 138 "intermediate" strains, 75 of which were contributed by workers outside this laboratory; and six strains which gave questionable VP reactions. This collection of cultures was considered representative of the groups in question.

¹ Original thesis submitted July, 1937. Doctoral thesis number 437.

The results of the fourth day of incubation showed that all strains tested utilized the nitrogen of adenine sulphate and xanthine. Since these compounds had no differential value, they were not considered further. The *Aerobacter* and VP questionable strains utilized the nitrogen of all other compounds tested, whereas a high percentage of the *Escherichia* utilized only the nitrogen of uracil, and the "intermediates" only the nitrogen of urea.

Vigor of growth was employed as the criterion of availability of the carbon, or both carbon and nitrogen, in the compounds studied. Observations with a limited number of strains indicated that of the compounds tested as carbon, or carbon and nitrogen sources, all but adenine sulphate supported a vigorous growth of *A. aerogenes*, but not *Escherichia*, *A. cloacae*, or "intermediate" strains. The adenine served as an available source of carbon, but not carbon and nitrogen, for *A. aerogenes* strains.

The test compounds (nuclei acid, uracil, allantoin, hydantoin, urea) were subjected to moderate autoclaving at 15 pounds for 15 minutes and the media cooled immediately. Subsequent inoculation with a limited number of organisms showed that of the compounds as nitrogen sources, that nucleic acid and uracil were not affected appreciably, but that allantoin, hydantoin, and urea were apparently modified by the heat treatment.

Investigations were carried out to test the possibilities of pyruvic acid fixation in a glycerol-peptone-bisulphite medium as a means of differentiating the "intermediate" strains from those belonging to the genera *Escherichia* and *Aerobacter*. Reynold's medium (1935) and the nitroprusside test for pyruvic acid (Simon and Piaux) were employed. It was found that both time and temperature had a decided effect on this test, and that due to the variability of results for the same organisms from day to day the establishment of standard conditions for a routine test would be difficult to attain.

A study of the individual reactions of the "intermediate" strains showed that: 83 per cent produced hydrogen sulphide (Vaughn and Levine medium); 94 per cent failed to produce indol from tryptophane; and that 95 per cent attacked cellobiose with production of either acid or acid and gas. A transfer of the original type species, *Citrobacter Freundii*, gave reactions typical of those given by a large proportion of the "intermediate" strains.

The VP questionable strains were allocated to the genus *Aerobacter*. The genus *Citrobacter* was accorded generic ranking with the genera *Escherichia* and *Aerobacter*.

REFERENCES

- REYNOLDS, H.
1935. The dissimilation of carbohydrates by the colon-aerogenes group of bacteria. Unpublished thesis. Library, Iowa State College, Ames, Iowa.
- SIMON, L. G., and L. PIAUX
1924. Sur le caracterisation et la dosage le petites quantites d'acide pyruvique. *Bul. de la Soc. Chem. Biol.* 6:477-486.
- VAUGHN, R., and M. LEVINE
1936. Hydrogen sulphide production as a differential test in the colon group. *Jour. Bact.* 32:65-73.

BACTERIOPHAGE SPECIFICITY FOR COLIFORM AND RELATED BACTERIA¹

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The specificity of bacteriophages for inducing lysis of different bacteria has not been extensively studied. It is generally recognized, however, that a certain degree of specificity does exist. d'Herelle (1) believes that some phages, when tested against members of the colon-typhoid group of bacteria, may attack only certain closely-related strains, but that other phages may attack bacteria in several different genera. Beard (2), using various methods, was unable to produce any adaptation of bacteriophages. Burnet and McKie (3) made extensive studies on the classification of phages, but have done only a limited amount of work on specificity. Evans (4) has employed bacteriophage to differentiate *Streptococci*. Krueger (5) reviewed some of the important contributions regarding bacteriophage.

The bacterial cultures against which bacteriophages were isolated and purified in this study include strains of *Escherichia*, "Intermediates," *Aerobacter*, *Proteus*, *Salmonella*, *Eberthella*, *Shigella*, *Alkaligenes*, *Klebsiella*, *Pseudomonas*, *Flavobacterium*, *Serratia*, and *Achromobacter*. In addition to the different strains for which phages against the foregoing bacteria were isolated, a large number of additional strains from the genera listed, particularly the coli-aerogenes group, were employed for studying the specificity of the bacteriophages.

The raw material for the isolation of the bacteriophages was sewage from the effluent of the Imhoff tank of the Ames sewage disposal plant and the effluent from the trickling filters of a packing house at Mason City, Iowa. For each bacteriophage isolated a preliminary enrichment tube was first prepared consisting of 10.0 cc. of broth, 1.0 cc. of the sewage and a light inoculum of the bacterial culture against which an homologous phage was desired. The enrichment tubes were incubated for 24 hours at 30° C. after filtration through sterile Chamberland-Pasteur L₃ filter candles and were then ready for testing and purification by plating procedures.

Petri dishes were prepared containing 15.0 to 20.0 cc. of nutrient agar of the following composition: 1.5 per cent Bacto peptone, 0.3 per cent beef extract, and 0.1 per cent agar. The plates were inoculated at the center with some bacteria from agar slants incubated 18 hours. The inoculum was smeared over the surface of the agar with a sterile, bent, glass smearing rod.

For inoculating the bacteriophages a 3.0 mm. platinum loop bent at an angle of 120° was used. One loopful of a phage filtrate was streaked successively across each petri dish prepared as described above. After incubation for 18 hours at 30° C. isolated phage plaques, usually of different size, appearance, and intensity, could be observed on the plates.

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Suitable, isolated plaques, together with some bacterial culture, were picked from each plate, emulsified in 10 cc. of broth, incubated, filtered, and replated, as just described. This procedure was repeated at least six times. Forty-three "pure" races of phage were obtained by this means. Twenty-eight of the 43 phages isolated were against coli-aerogenes strains.

In making specificity tests agar plates were prepared, inoculated, and smeared as before. Two phages were streaked on each plate. Each "pure" race of a phage employed for tests was inoculated onto a plate smeared with the culture to be tested for lysis. The phage was inoculated by making one streak about 2 inches long with the platinum loop. The plates were incubated for 18 hours and the results recorded. The test was considered to indicate positive lysis if no bacterial growth, or occasionally only partial bacterial growth, occurred in the area of the phage inoculum.

Two main series of specificity experiments were performed. In the first series 43 phages were tested against 94 coliform and related bacteria. In the second experiment 14 phages isolated against coliform bacteria were tested against 144 coliform organisms. The results indicate that:

There was a marked tendency to specificity of phages for the two main divisions of the coli-aerogenes group, as the following tabulation shows:

Cultures Tested	Phages Employed	Total No. of Tests	No. of Positive Tests	Percentage Lysed
20 <i>Escherichia</i>	10 " <i>Escherichia</i> "	200	47	23.5
20 <i>Aerobacter</i>	8 " <i>Aerobacter</i> "	160	47	29.4
20 <i>Escherichia</i>	8 " <i>Aerobacter</i> "	160	0	0.0
20 <i>Aerobacter</i>	10 " <i>Escherichia</i> "	200	1	0.5

The "Intermediates" show a moderate tendency to specificity of phages in the coli-aerogenes group, as the following indicates:

Cultures Tested	Phages Employed	Total No. of Tests	No. of Positive Tests	Percentage Lysed
20 "Intermediate"	8 "Intermediate"	160	46	28.7
20 <i>Escherichia</i>	8 "Intermediate"	160	13	8.1
20 "Intermediate"	10 " <i>Escherichia</i> "	200	11	5.5
20 <i>Aerobacter</i>	8 "Intermediate"	160	2	1.2
20 "Intermediate"	10 " <i>Aerobacter</i> "	200	2	1.0

The above figures also indicate, that on the basis of these bacteriophage tests, the "Intermediates" are more closely related to the genus *Escherichia* than to the genus *Aerobacter*.

No evidence of bacteriophage specificity was observed within the major sub-groups of the coli-aerogenes group of bacteria. There was no correlation between sucrose fermentation and susceptibility to lysis by

phage among the strains of the genus *Escherichia* or the "Intermediates," and no correlation between ability to ferment starch or to form indol from tryptophane and susceptibility to lysis by phage among the strains of the genus *Aerobacter*.

Genera for which phages appear to be specific include *Proteus*, *Flavobacterium*, *Serratia*, and *Klebsiella*. The remaining phages in this study (active against bacteria in such genera as *Escherichia*, *Aerobacter*, "Intermediates," *Salmonella*, *Eberthella*, *Shigella*, *Alkaligenes*, *Pseudomonas*, and *Achromobacter*) did not lyse members of the first four genera mentioned, except that a phage active against *Achromobacter lipolyticus* also lysed a strain of *Serratia indica*.

A reddish-orange, lactose-fermenting organism studied was more closely related to the coli-aerogenes bacteria than to the genus *Serratia* on the basis of phage tests. The organism was lysed by 2 *Escherichia* phages and 1 "Intermediate" phage, but no other phages, including 2 phages active against strains in the genus *Serratia*.

Some phages are limited in activity, attacking only coli-aerogenes strains, while others are active against a wider range of organisms (polyvalent phages), causing lysis of coli-aerogenes strains and also organisms associated with intestinal infections.

Many different races of phage appear to exist, as most of the 43 phages studied show different lytic specificities.

LITERATURE CITED

- BEARD, P. J.
1931. Bacteriophage adaptation. *J. Infect. Dis.* 49:367-398.
- BURNET, F. M., and M. McKIE
1933. The classification of the coli-dysentery bacteriophages. I. The differentiation by Bail's methods of phages lysing a typical *B. coli* strain. *J. Path. and Bact.* 36:299-306.
- D'HERELLE, FELIX
1926. *The Bacteriophage and Its Behavior*. Williams and Wilkins, Baltimore.
- EVANS, A. C.
1936. Studies on hemolytic streptococci. I. Methods of classification. *J. Bact.* 31:423-437.
- KRUEGER, A. P.
1936. The nature of bacteriophage and its mode of action. *Physiological Rev.* 16:129-172.

THE PRINCIPLE OF THE ARCHIMEDEAN SCREW IN CHEMICAL ENGINEERING¹

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The theory of the Archimedeian screw is developed, and the possibility of its application as a pump in chemical engineering, and the application of the principle by which it elevates liquids, to various chemical engineering operations, are investigated.

From the point of view of the chemical engineer the Archimedeian screw has a number of characteristics in its favor. It is completely valveless, essentially simple, and may be constructed of corrosion resisting materials. Although there are several modifications of the screw, the form which is considered in the investigation consists of a central cylindrical core on which are wound one or more vertical spiral flights, and an external cylindrical casing attached to the flights. The device is mounted at an angle with the horizontal so that the lower end dips into the liquid to be elevated, and is rotated about its axis.

The simple Archimedeian screw is open to the objections that it is extremely sensitive to the external liquid level, and unless conditions are very carefully controlled, performance is apt to be erratic and the efficiency low. It is shown, however, by an examination of the theory of the Archimedeian screw, that the low volumetric efficiency of the simple screw is due to the fact that as the screw rotates and successive slugs of liquid are picked up a partial vacuum is created in the air space between successive slugs. This action is cumulative, and eventually is sufficient to draw some or all of the liquid that has been carried part way up the screw back down the tube. This condition may readily be corrected, and high volumetric efficiency be obtained, by venting the air spaces between the liquid slugs to the atmosphere and thus equalizing the pressure throughout the screw.

The desired venting of the screw may be accomplished by any of several methods. Prior to this investigation, it was known that if the screw were operated in such a manner that the cross section of the spiral tubes was at no point completely filled with liquid, thus insuring a continuous air passageway through the screw, or if slots were cut in the core of the screw just below and following the lower surfaces of the several spiral flights, thus making it possible for air to flow in through the hollow core and balance the pressure, high volumetric efficiency was obtained. In either case, however, the quantity of liquid handled by a given screw was small. It is shown that much more liquid may be lifted if the screw is vented by a series of venting tubes in the first few turns of each of the spiral tubes, the venting tubes being inserted in holes in the casing and extending far enough up the casing parallel to the axis of the screw so that liquid will not flow out the open upper end. Or a slot may be cut in

¹ Original thesis submitted June, 1938. Doctoral thesis number 462.

the casing and the screw protected from loss of liquid through the slot by an external spiral sheet wrapped around and close to the casing, the lower edge of the sheet being attached to the lower edge of the slot. The quantity of liquid handled by a vented Archimedean screw is almost exactly the theoretical.

The capacity of the Archimedean screw as a pump is a function of the several variables in its design and the relationships of theoretical capacity to these variables are developed. The quantity of liquid handled by a vented screw, even when the lower end is completely immersed, varies directly as the speed of rotation. The capacity decreases with increasing angle of inclination, and is a function of the pitch of the flights. For each angle of inclination there is a specific pitch for maximum capacity, the optimum pitch decreasing with increasing inclination of the screw. The capacity is also a function of the ratio of core diameter to casing diameter, but the optimum ratio seems to be constant at one-half. The capacity of the screw is increased by the introduction of additional flights, but beyond four or five the slight gain in capacity does not warrant further addition.

The mathematical expression for the exact theoretical capacity of the Archimedean screw as a pump in terms of its design is quite involved. A graphical method by which the theoretical capacity may be closely approximated is developed. The graphical method is rather laborious, and a quicker, though under certain conditions somewhat less exact method, is developed on the assumption that the length of the wetted center line of the spiral tubes of the Archimedean screw is proportional to the volume of liquid in the tubes. By the use of the latter method the theoretical capacities of a number of screws were calculated and the above-mentioned relationships were developed.

Several small Archimedean screws were set up and tested, and the theories developed were confirmed.

If the height of the external spiral sheet, or "sidewall," above the flight of the vented screw mentioned above, be reduced as the external sidewall winds around the screw, liquid from within the screw will spill over the upper edge of the sidewall. If the reduction in height of the sidewall is gradual, the liquid will spill over its upper edge continuously as the screw rotates. It is shown that by attaching a continuous sheet fin to the upper edge of the sidewall the falling liquid may be distributed over a considerable area. The contour of the sidewall may be easily designed for any manner or rate of distribution of liquid over the fin.

A device such as outlined above would have the effect of lifting liquid through a small distance and at the same time distributing it over a large area, thus facilitating heat or gas transfer through the gas-liquid interface. A number of chemical engineering operations, notably aeration, gas scrubbing or absorption, stripping, heat transfer from gas to liquid or liquid to gas, and the like, are dependent upon obtaining as large an area as possible per unit volume of liquid, and a device of the type described should be applicable. Two such experimental "aerators" were set up and tested for rate of gas absorption and heat transfer, and the results, although inconclusive as yet, are sufficiently encouraging to warrant an extended investigation of the possibilities of the device.

PASSAGE-TIME OF VARIOUS TYPES OF NORMAL AND POISONED FOODS THROUGH THE ALIMENTARY TRACT OF THE COCK-ROACH *PERIPLANETA AMERICANA* LINN¹

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Investigations were conducted to determine rates of passage of some normal and poisoned foods through the alimentary tract of the cockroach, *Periplaneta americana* Linn. Approximately 700 adults and last instar nymphs were dissected to determine progress of dyed material along the digestive canal at various intervals after ingestion; about 900 were tested for total passage-time (designated as egestion time) by means of mechanical excrementometers devised for this purpose. These mechanisms are figured and described in detail. All insects were starved for 48 hours before beginning tests. Only normal active specimens were used. Experimental animals were permitted access to water throughout the trials.

Banana paste or commercial Purina chow formed the base of all test meals used. Five per cent powdered carmine dye was added to each preparation to render test material identifiable in the digestive tract and in the fecal pellets. All test meals were slightly sweetened by addition of 1 per cent finely pulverized cane sugar. Passage-times of the 13 following test meals were determined: banana paste, desiccated banana, banana-barium sulphate, banana-sodium fluoride, banana-phenothiazine, banana-arsenate of lead, banana-astringent arsenate of lead, banana-arsenic trioxide, banana-rotenone, Purina powder, Purina-barium sulphate, Purina-sodium fluoride, and Purina-phenothiazine.

Figures are included to illustrate average rates of progress of these test meals and tables list egestion time records of all populations used.

First ingested portions of banana paste pass through the stomodaeum in less than one-half hour, complete passage along the mesenteron and enter the pylorus in 2.5 to 3 hours, require from 5 to 6 hours in the anterior intestine, and remain 10 to 12 hours in the rectum. It seems quite evident that portions of the anterior intestine and rectal epithelium function in absorbing considerable liquid from semi-fluid material present in the lumen of the proctodaeum. Total passage-times for banana paste residues averaged 20.22 ± 6.9 hours. Repeated trials with the same test insect demonstrated considerable inconsistency in individual egestion times.

Rate of progress of Purina powder is quite similar to movement of banana paste along the stomodaeum and mesenteron. From 3.5 to 4 hours are required for passage of the Purina residue through the anterior intestine; rectal retention time is normally about 14 hours. Mean egestion time for indigestible residue of this material was 21.45 ± 6.93 hours.

Mean egestion time of desiccated banana was 13.48 ± 3.79 hours. Average elimination period of banana-barium sulphate residua was 15.58 ± 6.47 hours. Velocity of passage along the anterior intestine was

¹ Original thesis submitted July, 1937. Doctoral thesis number 443.

considerably increased. Mean egestion time for Purina-barium sulphate waste was 20.03 ± 7.83 hours. Sodium fluoride caused great decreases in rate of movement through the anterior intestine. Mean egestion times for banana-sodium fluoride and Purina-sodium fluoride were 30.99 ± 11.75 hours and 28.67 ± 9.21 hours, respectively. Phenothiazine with banana paste produced a decrease of about 2 hours in the period required in the anterior intestine. Egestion times for residua of this material averaged 18.05 ± 7.78 hours. Mean total passage-time for Purina-phenothiazine waste was 21.42 ± 7.48 hours.

Banana-arsenate of lead preparations (with or without astringent) showed somewhat greater velocity of movement through the anterior intestine. Mean egestion records for lead arsenate and astringent lead arsenate with banana paste were 18.08 ± 8.66 hours and 17.57 ± 7.09 hours, respectively. Arsenic trioxide with banana paste caused noticeable destruction of ventricular tissue and greatly decreased rate of progress through the anterior portion of the proctodaeum. Distribution of this material in the mesenteron was irregular as greater concentrations usually occurred at the cardiac and pyloric ends. Mean time of evacuation of first portions of this food-poison residuum was 28.91 ± 5.81 hours. Rotenone with banana caused a decrease of 2.5 hours in time necessary in the anterior intestine. Mean egestion time for indigestible residue of this material was 18.24 ± 5.97 hours.

Records of mortality produced by food-poison mixtures during a 50 hour observation period are listed. Highest kill (53 per cent) was recorded with banana-arsenic trioxide.

Normal velocities of food movement in man and this insect are physiologically comparable. In man food material begins to leave the stomach immediately after ingestion, passes rapidly through duodenum and jejunum, moves progressively slower in the ileum as the ileocaecal valve is approached, is retained in the colon from 12 to 14 hours, and is normally evacuated from 16 to 24 hours after entering the stomach. A decrease in velocity of food movement along the alimentary tract of the roach is evident in the vicinities of the mesentero-proctodael junction and the intestino-rectal constriction. Comparison of these relationships does not infer morphological similarity.

In addition to changes in rate of progress produced by variations in chemical and physical characters of the food, the following factors were investigated to determine their effects on velocity of food movement: sex, stage of development, amount of material ingested, length of starvation period before feeding, size of previous meal, excitement or undue disturbance, and amount of general muscular activity.

Sex and stage of development (limited to adults and nymphs in last instar) were not found to influence velocity of food movement. The following factors were found to increase rate of food progress: ingestion of larger amounts of unpoisoned preparation (measured by degree of crop distention), undue excitement or fright, longer periods without food before testing, and general increase in muscular activity. Decreases in velocity of movement of sodium fluoride and arsenic trioxide mixtures were correlated with the amount of food-poison ingested. Velocity of progress was decreased by the presence of large amounts of a previous meal in the stomodaeum.

STUDIES ON LACTOBACILLUS CULTURES THAT ACTIVELY COAGULATE MILK¹

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The primary object of the work herein reported was to determine the general relationships of cultures of Lactobacilli that actively coagulate litmus milk with reduction of the litmus and the formation of a smooth curd. Most of the cultures were isolated from dairy products and materials such as ensilage, corn stover and feces from calves, dogs, rats and infants. Cultures of *L. acidophilus* and *L. bulgaricus* were obtained from various laboratories for comparative purposes. A total of 36 cultures was studied, particularly from the standpoint of their action in milk because of the importance of the organisms in dairy products.

In a comparison of the value of various solid media for isolating Lactobacilli, it was found that tomato juice agar gave higher and more uniform counts than beef infusion agar plus 1 per cent lactose, cabbage agar, casein digest agar or whey agar; the results obtained with beef infusion agar plus lactose were strikingly lower than the counts obtained with the other media. The isolation of Lactobacilli from dairy products was facilitated by incubating the material being examined in milk until the microflora showed a predominance of characteristic rod forms, and then plating on tomato juice agar. Nutrient broth containing 2.0 per cent dextrose and 5.0 per cent N/10 acetic acid was useful as an enrichment medium in isolating Lactobacilli from fecal material.

Appreciable quantities of total acid, volatile acid and acetylmethylcarbinol plus diacetyl were produced in milk by most of the 36 cultures of Lactobacilli studied but the amounts of these materials produced by the different cultures varied greatly and there was no close correlation between the amounts of total acid and the amounts of volatile acid or acetylmethylcarbinol plus diacetyl produced by a culture. The addition of citric acid or acetaldehyde to milk cultures did not significantly influence the production of volatile acid or of acetylmethylcarbinol plus diacetyl by the organisms. Low concentrations of the acetaldehyde resulted in slight increases in the amounts of acetylmethylcarbinol plus diacetyl produced while higher concentrations appeared to be toxic to the organisms.

Most of the cultures studied produced appreciable quantities of carbon dioxide in skim milk; the values were generally lower when peptone was added to the milk. More carbon dioxide was produced at 37° C. than at 21° C. during incubation for 15 days.

The fermentation reactions obtained with the Lactobacilli studied were too variable to be used as criteria for the separation of the cultures into species. Dextrin, dextrose and lactose were the carbohydrates most commonly attacked. None of the organisms that grew on Nile-blue sulfate agar hydrolyzed butter fat.

¹ Original thesis submitted June, 1936. Doctoral thesis number 386.

The isomeric form of lactic acid produced was determined with 30 of the cultures. The form of acid varied from pure active to nearly pure inactive and was usually dextro-rotatory. There was apparently no relation between the types of lactic acid produced and the sources of the organisms. Most of the cultures of *Lactobacilli* were able to break down the proteins in milk as indicated by increases in amino nitrogen content during incubation at 37° C. for 8 weeks. Larger amounts of amino nitrogen were produced when the acid developed was partially neutralized with calcium carbonate.

The addition of 0.13, 0.7 or 5.0 per cent of deuterium oxide to whey broth cultures of *Lactobacilli* had no influence on the morphology or rate of multiplication of the organisms.

Evidence was obtained which indicated that the growth temperatures may be useful in classifying the *Lactobacilli*. All the organisms grew well and coagulated milk at 37° C. but the results obtained at the other temperatures were variable. The *L. acidophilus* and *L. bulgaricus* cultures obtained from various research laboratories and the cultures isolated from fecal material grew well at 37° and 45° C. but not at 10° or 21° C. In general, the *Lactobacilli* from raw milk, Swiss cheese, Cheddar cheese and feed grew at 21° C. but not at 45° C.; a few of these organisms also grew at 10° C.

None of the *Lactobacilli* produced pronounced flavors in milk other than acid, which varied from mild and desirable to sharp, high acid and undesirable. The odor produced in milk by the *Lactobacilli* suggested acid and varied from none to very strong. Cultures that produced a clean, mild acid flavor usually gave a clean, desirable acid odor, while the cultures that produced an unclean, flat acid flavor gave an unclean odor. There appeared to be no relation between the sources of the organisms and the flavors and odors produced in milk.

In isolating the *Lactobacilli*, organisms were encountered which resembled *Lactobacilli* very closely but which did not coagulate milk rapidly. This suggests that not all *Lactobacilli* coagulate milk rapidly or that there is a group of closely related organisms which do not grow well in milk.

THE RING STRUCTURE IN SOME DERIVATIVES OF SORBOSE¹

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The assignment of ring configuration is of first importance in developing the chemistry of sugar derivatives. With the exceptions of diacetone sorbose (1) and the open chain pentacetate (2, 3) the ring structures for *l*-sorbose and its derivatives have, up to this time, remained undetermined.

Since α -methyl-*l*-sorbose was one of the earliest known derivatives of sorbose, and since the glycosides are important central compounds in the sugar series, the determination of ring configuration for α -methyl-*l*-sorbose was undertaken. α -Methyl-*l*-sorbose was methylated to a pentamethyl derivative, which was hydrolyzed to a tetramethyl-*l*-sorbose (4). Nitric acid oxidation of this latter compound resulted in the production of dextro dimethoxysuccinic acid in good yield. The presence of this acid was established through the preparation of its amide and methylamide. These are well-defined crystalline derivatives prepared by Haworth (5) as reference compounds in the sugar series. The isolation of dextro dimethoxysuccinic acid asserted the methylation of carbon atoms four and five in the tetramethyl-*l*-sorbose, since no other adjacent carbon atoms could give rise to an acid of this configuration. Hence, the lactol ring must have engaged either carbon atom three or carbon atom six in the tetramethyl sorbose. As ethylene oxide rings are not known to form under the conditions employed for glycoside formation, the possible existence of such a ring was not considered. This left only a six membered, or normal pyranoid ring structure, to be assumed for the tetramethyl-*l*-sorbose and, hence, also for α -methyl-*l*-sorbose and its derivatives.

When *l*-sorbose was treated with dry ethanol containing one per cent hydrogen chloride, α -ethyl-*l*-sorbose was produced. This compound crystallized as fine colorless needles having a melting point of 116° and a specific optical rotation in water of $[\alpha]_D^{26} - 73.9^\circ$. Acetylation of this substance produced α -ethyl-*l*-sorbose tetraacetate having a melting point of 75° and a specific optical rotation in chloroform of $[\alpha]_D^{26} - 54.6^\circ$. This α -ethyl-*l*-sorbose tetraacetate was identical with that obtained through the ethylation of sorbose tetraacetate. Thus, the ring structure of α -ethyl-*l*-sorbose must be the same as that in sorbose tetraacetate. The structure of sorbose tetraacetate as 1,3,4,5-tetraacetyl sorbose can be established through the fact that acetylation of α -methyl-*l*-sorbopyranoside, whose configuration was proven as indicated above, yields an α -methyl-*l*-sorbose tetraacetate identical with the compound obtained through the methylation of sorbose tetraacetate. This series of reactions indicated a normal pyranoid ring structure for α -ethyl-*l*-sorbose and its tetraacetate.

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In comparing the rates of formation of α -methyl-*l*-sorbopyranoside and α -ethyl-*l*-sorbopyranoside it was found that the reactions were essentially complete in four hours, when the *l*-sorbitose was dissolved in either methanol or ethanol containing one per cent hydrogen chloride and the mixture allowed to stand at room temperature.

The rates of hydrolysis for α -methyl-*l*-sorbopyranoside and α -ethyl-*l*-sorbopyranoside were also found to be almost identical. Dissolved in 0.015 normal hydrochloric acid both glycosides were hydrolyzed in about thirty days when kept at a temperature of 30°. It was interesting to note in this connection that these rates of hydrolysis did not differ widely from the rate found for methyl-*d*-fructofuranoside (6).

Calcium chloride was known to form crystalline addition compounds with several sugars. The preparation of a calcium chloride compound of *l*-sorbitose was undertaken in the hope of obtaining a compound of sorbitose in either of its alpha or beta stereoisomeric forms. When *l*-sorbitose and calcium chloride in equal molar proportions were dissolved in water and the solution allowed to evaporate slowly, fine crystals of a calcium chloride addition compound of α -*l*-sorbitose were obtained. After purification the crystals showed a melting point of 159° (Corr.) and a specific optical rotation in water of $[\alpha]_D^{20} - 24.2^\circ$. This compound exhibited a rapid but small upward rotation. In fifteen minutes the value became constant at $[\alpha]_D^{26} - 23.9^\circ$. The compound gave the following formula on analysis: $C_6H_{12}O_6 \cdot CaCl_2 \cdot 2H_2O$. On acetylating the addition compound by the general procedure for acetylation there was produced in good yield keto sorbitose pentaacetate. Hence, the property of *l*-sorbitose to form preferentially an open-chain pentaacetate is also exhibited by the calcium chloride addition compound. Acetylation, using the special method designed to give sorbitose tetraacetate, produced this tetraacetate in good yield. These facts indicate that calcium chloride does not stabilize sorbitose in a new form.

By means of hydrogenation and acetylation followed by the subsequent isolation of the hexaacetate of *d*-sorbitol and *l*-iditol, Cramer and Pacsu (3) have proven that the common pentaacetate of *l*-sorbitose is a derivative of the open-chain sugar. This conclusion might have further been substantiated if it could have been shown that sorbitose pentaacetate were capable of mercaptalization to yield *l*-sorbitose ethyl thioacetal pentaacetate. Following the method advanced by Wolfrom (7) for the mercaptalization of fructose, the mercaptalization of keto sorbitose pentaacetate was carried out. The product obtained was a colorless sirup which could not be crystallized. The sirup showed no reducing properties when tested with Fehling's solution. The sirup had a refractive index of $N_D^{24} 1.5030$ and specific optical rotation in chloroform of $[\alpha]_D^{27} - 12.2^\circ$. The substance distilled in a high vacuum and at a bath temperature of 200° to produce a sirup having a refractive index of $N_D^{24} 1.5050$ and a specific optical rotation in chloroform of $[\alpha]_D^{30} - 13.1^\circ$. The distillate was light brown in color showing that a slight decomposition had occurred during the distillation. Neither the original sirup nor the liquid distillate showed any evidence of mutarotation.

LITERATURE CITED

- (1) REICHSTEIN AND GRUSSNER
1934. *Helv. Chim. Acta*, 17, 311.
- (2) SCHLUBACK AND VORWECK
1933. *Ber.*, 66, 1251.
- (3) CRAMER AND PACSU
1937. *J. Am. Chem. Soc.*, 59, 1467.
- (4) ARRAGON
1936. *Bull. soc. chim. biol.*, 18, 1336.
- (5) HAWORTH AND JONES
1927. *J. Chem. Soc.*, 2349.
- (6) MENZIES
1922 *Ibid.*, 2238.
- (7) WOLFROM AND THOMPSON
1934. *J. Am. Chem. Soc.*, 56, 880.

STUDIES ON THE PHYSICAL-CHEMICAL PROPERTIES OF HONEY¹

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DeBoer and Knipphorst (1932) found that a heather honey exhibited the gel/sol transformation brought about by stirring which has been termed thixotropy by Freundlich (1928). Scott Blair (1935) observed the phenomenon in another heather honey, as did Pryce-Jones (1936a). Honeys from sources other than heather were found to be non-thixotropic. Convincing evidence has been advanced by Pryce-Jones to show that colloidal materials in heather honey are responsible for its unique behavior.

Paine, Gertler and Lothrop (1934) have investigated a second property of honey, closely related to thixotropy, that of anomalous viscosity. The apparent viscosity of buckwheat honey was found to vary with the shearing force involved in the measurements. The anomalous behavior disappeared when the colloidal material was removed by ultrafiltration.

The accurate measurement of thixotropy is difficult; the only means available, in many cases, of following the gel/sol transformation is to make observations of the apparent viscosity before and after stirring. Pryce-Jones (1936b), however, has claimed that all viscometric methods so far proposed are subject to error. There are two obstacles inherent. First, a certain amount of stirring is involved in the measurement of the viscosity. This tends to lower the apparent viscosity and the lowering will be different for various observations. Second, viscosity determinations on the sol and gel forms, which may differ widely in apparent viscosity, are not readily made at the same shearing force. Since the apparent viscosity of each form is a function of the shearing force, comparisons of the apparent viscosities have little meaning unless this variable is held constant.

The falling sphere viscometer was chosen for this research since it has found wide use in such studies and because it seemed to offer certain advantages over other instruments. The stirring actions of the smallest spheres is practically negligible and by proceeding from small to large spheres in the measurements it is possible to obtain the curve, apparent viscosity against shearing force, for the undisturbed gel. The same curve is readily obtained for the sol form, on which stirring has no effect, and comparison at the same shearing force can be made from the two curves. A standard shearing force of zero was selected at which comparisons were made. Apparent viscosities η , were calculated from the velocity of the sphere by the following equation,

$$\eta = \frac{2(d_2 - d_1)gr^2f_v}{9V} \quad (1)$$

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This is the Stokes' law equation multiplied by f_w , the wall correction factor of Faxen (1922). Apparent viscosities were plotted against sphere radius and an extrapolation made to zero sphere size, that is, to zero shearing force. The number of observations made in fixing the curve for the gel form was limited as much as possible to avoid stirring. The honey was then stirred with a rotary stirrer and a second series of observations was made. The numerical value of the thixotropy was taken as the change in apparent viscosity in poises, at zero shearing force, which occurred on stirring. Since thixotropy changes with time and since the rate of change depends on the size of the containing vessel, it was necessary to fix arbitrary conditions under which the thixotropy of the honey was measured. The honey was heated for one hour at 60°, poured into a fall cylinder of 2.2 centimeters radius and allowed to cool to room temperature. The cylinder was then placed in the thermostat and the honey was maintained at 25° for 48 hours, at which time the measurements were made. The thixotropy of a number of Iowa honeys was measured and found to vary from 3 to 15 poises.

In order to rate the anomalous viscosity it was necessary to find the shearing force involved in each measurement. The equation of Lamb (1906) has heretofore been used by viscometrists to calculate the shearing force in the falling sphere viscometer. This equation is

$$F = \frac{3\eta V}{2r} \quad (2)$$

where η is the apparent viscosity, V the velocity of the sphere and r its radius. Preliminary work with honey, however, indicated equation (2) to be incorrect. An equation for the same purpose has recently been advanced by Pasynskii and Rabinovich (1934). This is

$$F = \frac{\eta V}{R - r} \quad (3)$$

where R is the radius of the fall cylinder and the other symbols have their previous meanings. Equation (3) gave indications of being valid. Honey, however, is a liquid definitely unsuited to use as a test liquid to decide between the two equations. For this purpose data are required which have been obtained with a variety of fall cylinders and spheres; thixotropic setting is most pronounced in a small vessel and a honey after standing in several fall cylinders would not be in a comparable state in each. The data available in the literature were considered and those of Sheppard (1917) on a nitrocellulose solution were chosen. The equation of Pasynskii and Rabinovich was found to give an excellent representation of the data, while that of Lamb did not. It is felt that the equation of Pasynskii and Rabinovich, apparently not available outside of a Russian publication until recently, is an important contribution to the theory of the falling sphere viscometer; it places this viscometer on an equal footing with capillary and rotating cylinder instruments for the study of anomalous systems. A further advantage of equation (3) is that data from the falling sphere viscometer and the capillary tube instruments on

plotting fall on a common apparent viscosity-shear force curve. This was not necessarily to be expected.

In several honeys the apparent fluidity, ϕ , was found to increase with the shearing force, F , according to the Bingham-Williamson equation,

$$\phi = a - b F \quad (4)$$

where a and b are constants. This equation is discussed in detail by Bingham (1928). In one honey, however, there was found a superimposed tendency to behave in an opposite manner, that is, the fluidity decreased with increasing shear force. This seemed to be the *elastic recoil* observed by Pryce-Jones (1936a), as far as such a property could be identified in a dissimilar instrument. The possibility of the presence of *elastic recoil* makes the rating of the anomalous viscosity uncertain. It was therefore decided not to attempt a numerical rating of this property. It is of interest, however, that each of the honeys observed showed an apparent viscosity which varied with the shearing force.

The honeys were analyzed for the water content by the refractometric method of Fulmer, Bosch, Park and Buchanan (1934) and the nitrogen content was determined. The pectin content was estimated by a modification of the method of Carré and Haynes (1922). A preliminary acidification with acetic acid, boiling and filtering was introduced to prevent the precipitation and weighing of protein as pectin. The volume of protein precipitated from 5 grams of diluted honey by phosphotungstic acid was determined. While Pryce-Jones found thixotropy only in honeys giving 30 cc. of precipitate, each of the honeys observed was definitely thixotropic and the volume of precipitate was never more than 0.3 cc. The lack of agreement is probably only apparent, since the heather honeys investigated by Pryce-Jones have anywhere from 200 to 2,000 times as much thixotropy as the honeys dealt with here.

Other investigators have found a general correlation between thixotropy and the colloidal content of the honey. The heather honey, for which thixotropy had been reported, contained up to 6 per cent of such materials. The colloidal content of the honeys studied in this thesis was less than 1 per cent and the observed thixotropy was much lower than that observed in heather honey. It is not surprising, therefore, that no definite relationship was found between the colloidal content and thixotropy. However, an invert sugar solution did not show thixotropy. It may be inferred, therefore, that the low thixotropy of the honey is associated with the colloidal material present.

LITERATURE CITED

- BINGHAM, E. C.
1928. Colloid types. Colloid Symposium Monograph 5:219-228.
- CARRÉ, M. H., and D. HAYNES
1922. The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. J. 16:60-69.
- DEBOER, H. W. and L. C. E. KNIPHORST
1932. Thixotropie van Heidehoning. Chem. Weekblad 29:526-534.

- FAXEN, H.
1922. Die Bewegung einer starren Kugel längs der Achse eines mit zäher Flüssigkeit gefüllten Rohres. Arkiv Mat. Rstron. Fysik 17, No. 27, 1-28.
- FREUNDLICH, H.
1928. Ueber thixotropie. Kolloid-Z. 46:289-299.
- FULMER, E. I., W. BOSCH, O. W. PARK, and J. H. BUCHANAN
1934. The analysis of the water content of honey by use of the refractometer. Am. Bee J. 12:208-212.
- LAMB, H.
1906. Hydrodynamics. University Press, Cambridge.
- PAINE, H. S., S. I. GERTLER, and R. E. LOTHROP
1934. Colloidal constituents of honey. Ind. Eng. Chem. 26:73-81.
- PASYNISKI, A. G., and A. I. RABINOVICH
1934. The viscometry of highly viscous lyophilic colloids. II. Application of viscometers based on Stokes' law. (Translated title.) Zhur. Fiz. Khim. Leningr.) 5:521-533.
- PRYCE-JONES, J.
1936a. Thixotropy and elastic-recoil in heather honey. Bee World 17:89-94.
- PRYCE-JONES, J.
1936b. Some fundamental aspects of thixotropy. J. Oil and Colour Chem. Assoc. 19:295-337.
- SCOTT BLAIR, G. W.
1935. The thixotropy of heather honey. J. Phys. Chem. 39:213-219.
- SHEPPARD, S. E.
1917. The measurement of the absolute viscosity of very viscous media. Ind. Eng. Chem. 9:523-527.

CHARACTERISTIC FEATURES OF THE MICROBIOLOGY OF THE WEBSTER AND TAMA SILT LOAMS¹

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Since the recognition of the existence of a microbiological population in the soil and the demonstration that the activities of the microorganisms concerned have a great influence on soil fertility, constant attempts have been made to construct some picture of the distribution of the soil population and to understand the relationships between the various groups of organisms composing it. Attempts to obtain an index of the microbiological condition of the soil by the determination of those biochemical changes that the population could bring about have in most cases been recognized as being artificial. The well-known plate and dilution methods have given interesting and valuable data on the types of organisms present and their individual potentialities but have added little to the study of the population as a whole. However, increasing success is following the more straightforward approach of direct examination, now that some of the many serious technical difficulties have been overcome.

This investigation was an attempt to follow by direct examination, supplemented by field microscopic observations, the characteristic features of the microbiology of two typical Iowa soils.

Both soils used in this study, a Tama silt loam and a Webster silt loam, are widely distributed upland types in their respective areas. The Tama silt loam, found in the Mississippi loess area, has a pH value of about 5.6 and the Webster silt loam, which is typical of the Wisconsin drift area, has a pH of about 6.6.

Since it is known that the addition of available organic matter to soils greatly influences microbial activity it was believed any fundamental microorganic differences between the two soils under investigation might be brought out or emphasized in their behavior on addition of green manure. In addition to the examination of the soils themselves, observations were therefore made on samples into which soybeans and alfalfa had been incorporated as green manure. Such work calls for methods which do not destroy the natural soil structure. Only two of the direct methods considered fulfill this requirement. One is the method of Kubiěna and the other the Rossi-Cholodny buried slide technique. A third method, the so-called "bodenstaub" technique proposed by Cholodny, and a modification of it, although involving destruction of the initial soil structure, was also used.

It was learned that during wet periods anaerobic decomposition of plant remains took place in the depressed areas of the Webster silt loam whereas in better drained areas the decomposition was aerobic. This was evidenced by the soggy condition of the cornstalks taken from below the soil surface in the former areas and the lower moisture content of those

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from the latter areas. Numerous mites and protozoa were seen in the pithy region of the soggy stalks.

Laboratory observations of the Kubiëna cultures revealed that well decomposed farmyard manure failed to cause any appreciable mycelial development in the Tama soil, whereas the addition of soybeans and alfalfa stimulated the development of an abundance of mycelium in both the Webster and Tama silt loams. An interesting feature of this increase was the succession of the fungi, which, though not identical in both soils nor in one soil with both additions, had distinct resemblances. Microscopic examination of plant residues taken from the Tama soil showed an abundance of actinomycetes. These microorganisms were also numerous in the Kubiëna cultures of this soil.

Apart from the succession of the fungi other interesting features brought out by the Kubiëna cultures were the differences between the soil forms and culture forms of *Rhizopus*, and the different fruiting habitats of *Aspergillus* and *Rhizopus*.

A noticeable difference between the two soils was revealed in the Kubiëna cultures which had received alfalfa and soybeans as additions. In each soil during the first few days there was a rapid development of fungi, possibly more in the Webster than in the Tama, this being followed by a decrease in abundance of new hyphae. The decrease was slow and extended over a considerable period of time in the Tama soil, while in the Webster soil there appeared to be an almost complete cessation of fungous development after the seventh to the twelfth day. The explanation for this difference may lie in the physical characteristics of the two soils.

The information acquired from the buried slides was practically the same as that from the Kubiëna cultures, except that the observation of bacteria was possible and their distribution could therefore be followed. Some groups of bacteria apparently were concerned more with the breakdown of previously elaborated fungous tissue than with that of the plant additions.

The "bodenstaub" technique and its modifications failed to give any data comparable to either the Kubiëna cultures or the Rossi-Cholodny technique.

As far as the suitability of the various methods employed is concerned, that of Kubiëna seems to offer the greatest possibilities, particularly with respect to fungi. The results given by this technique were in accord with those obtained from the field investigations. The Rossi-Cholodny procedure is in reality a field method and it does provide related information supplementing that obtained from the Kubiëna cultures. It also gives a better picture of the bacteria than any of the other methods used in this study. The "bodenstaub" technique and its modification destroys the natural structure of the soil and therefore the conditions are so changed that the organisms developing may not truly represent the natural flora. The modification of using fresh soil particles containing plant residues is capable of giving much useful information, particularly with respect to the soil protozoa, the development of which is probably favored by the high moisture conditions.

The results of this work may be summarized as follows:

1. The Webster and Tama silt loams are alike in that in each the fungous activity was increased when green manure was added to the soil.
2. In each soil a definite succession of fungi followed the addition of green manure.
3. Most of the decomposition taking place in the Webster soil is normally anaerobic in wet periods except at the very surface.
4. *Aspergillus* and *Rhizopus* showed a difference in fruiting habitats.
5. The soil form of *Rhizopus* differed from the culture form with respect to the number of sporangiophores.
6. Certain groups of bacteria seemed to be almost entirely engaged in the decomposition of fungous tissues in the soil.
7. The numerous mites found in the pithy region of the cornstalks buried in the Webster silt loam indicated that these invertebrates may be important in the decomposition of plant residues.
8. Laboratory methods, such as the Kubiëna technique, which do not completely destroy the initial structure of the soil, more nearly simulate field conditions and yield information comparable to that obtained by field investigations.
9. The Rossi-Cholodny method offers a means of studying the influence of materials, such as green manure, added to the soil and at the same time preserving the natural habitats of the organism acting upon such materials.
10. The "bodenstaub" procedure and its modification have such limitations that their value as direct methods of soil investigation are questionable, although they may be used in protozoan studies.

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