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A LIST OF IOWA ANTS[1]

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Since the publication of the author's preliminary list of Iowa ants (1941a), extensive collecting in many parts of Iowa has made possible a more complete list, comprising 97 forms. Five of these forms are new to science.

The writer believes that the listed forms comprise a large percentage of the ants which exist in Iowa. Ten or perhaps even 20 additional species might be collected. To do so, however, would take years of intensive collecting and a systematic survey of every county. No such survey was possible for the writer. Instead, an attempt was made to collect in all the different ecological areas, and to find as many good collecting areas as possible. In the search for the latter the numerous state parks of Iowa proved very helpful. Backbone State Park in Delaware County deserves particular mention. At this park a new and extraordinary species, *Lasius (A.) plumopilosus* Buren, was found, along with several other species rare or lacking in other parts of the state.

In general there are only two main faunal areas in Iowa. The first and by far the largest may be termed the Mississippi area. It occupies the large portion of Iowa within the Mississippi River drainage system. It is characterized by an ant fauna much like that of the states farther east. The genera richest in species are *Formica* and *Lasius,* and to a lesser extent *Camponotus, Leptothorax, Aphaenogaster,* and *Myrmica.*

The second area is much smaller, comprising only the bluffs along the Missouri River. These bluffs consist of loess soil and are very steep and quickly drained, ecologically simulating the arid southwestern states or the Great Plains. This condition is reflected in the ant fauna. *Eciton* and *Iridomyrmex,* two genera found in these bluffs, are not represented in the rest of the state. Also found in this region are *Pheidole sitarches* Wheeler, *Paratrechina (N.) arenivaga* Wheeler, *Dorymyrmex pyramicus* (Roger), *Crematogaster minutissima missouriensis* Emery, *Camponotus caryae rasilis* Wheeler, and *Formica pallidefulva dolosa* Wheeler, species not found in the Mississippi area.

The area drained by the rivers and streams which flow into the Missouri River seems transitional between the Mississippi River drainage

[1] Revised from "A monograph of Iowa ants (Formicidae, Hymenoptera)," an unpublished thesis submitted to the graduate faculty of Iowa State College for the degree Master of Science.

[2] The author is deeply indebted to Dr. H. H. Knight, Professor of Entomology, Iowa State College, and to Dr. M. R. Smith, Associate Entomologist, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, for constant encouragement, advice, and criticism on all phases of the work.

area and the Missouri River bluff area, and contains elements of both faunas.

At least two species, *Aphaenogaster treatae* Forel and *Ponera trigona opacior* Forel, have a discontinuous distribution, being found only in the bluffs of the Mississippi and Missouri rivers. These are southern species which appear to have crept northward only along the large rivers.

Two species, *Pogonomyrmex occidentalis* (Cresson) and *Formica fusca neoclara* Emery, were found in the Iowa State College collection labeled Sioux City, Iowa. Since both these species have heretofore been known only from the Great Plains and Rocky Mountains, their existence in Iowa should remain in doubt until validated. If these two species do occur in Iowa, they belong to the Missouri River bluff fauna.

In the following list, the writer has recognized only one infraspecific variant, the subspecies, in contradistinction to most of the older, and many of the recent, authors who recognize both subspecies and varieties. For as Creighton has shown (1938), there is no valid difference between the subspecies and the variety, and the retention of both ranks only complicates the nomenclature. The author has, therefore, raised all forms in this list formerly considered as varieties to subspecific rank. In some instances the raising of certain varieties to subspecific rank seems to cast doubt on their validity, and the author has discussed their taxonomic status.

Creighton states (1938) that all variants are probably geographical races. It seems likely, however, that many variants are ecological races rather than geographical ones. One race, for instance, may live in woodland, while another may prefer prairie or open fields. Such variants may occur in the same locality with little intergradation. An example of this is *Myrmica sabuleti trullicornis* n. subsp., a woodland form, whose closest relative is *Myrmica sabuleti americana* Weber, a prairie form.

No forms or records have been included in the list unless the specimens were seen and studied by the author. All collections were made by the author unless otherwise stated. Holotypes of the new species are in the author's collection, and paratypes will be given to the National Museum, Iowa State College, and other institutions and individuals.

For entomologists who may wish to identify Iowa ants, keys for the separation of all forms listed are included.

Key to Subfamilies of Formicidae

1. Pedicel of abdomen 2-jointed 2
   Pedicel of abdomen 1-jointed 3
2. Frontal carinae usually somewhat separated and at least partially covering the antennal insertions ..... 3. *Myrmicinae*
   Frontal carinae approximate, linear, not at all covering antennal insertions ..... 2. *Dorylinae*
3. A distinct constriction between the first and second segments of the gaster; sting developed ..... 1. *Ponerinae*
   Without a constriction between first and second segments of gaster; sting vestigial or absent 4

4. Apex of hypopygium with a circular, hair-fringed opening[3] for the ejaculation of the poison .....5. *Formicinae*
Hypopygium without such an opening .....4. *Dolichoderinae*

1. SUBFAMILY PONERINAE

KEY TO GENERA OF PONERINAE

1. Mandibles falcate, their teeth bifurcated.....1. *Stigmatomma*
(one Iowa species, *S. pallipes subterranea* Creighton)
Mandibles and their teeth normal.....2
2. Tip of gaster strongly deflected ventrally and anteriorly, petiole nodiform .....2. *Sysphincta*
(one Iowa species, *S. pergandei* Emery)
Tip of gaster not bent anteriorly underneath; petiole with a large erect scale .....3. *Ponera*

1. Stigmatomma

1. *Stigmatomma pallipes subterranea* Creighton

1940 *S. pallipes subterranea* Creighton, Amer. Mus. Nov., No. 1079:8. ☿

Records: Ames, Burlington, Bellevue. Also Sioux City (C. N. Ainslie).

The writer has taken this form under rocks in woodlands at Burlington and Bellevue and in an open field at Ames. It can be found only in the spring or fall when the ground is moist. Never more than seven specimens were found at one time.

2. Sysphincta

1. *Sysphincta pergandei* Emery

1895 *S. pergandei* Emery, Zool. Jahrb. Syst., 8:264. ☿

Record: Bellevue.

Apparently this species is extremely rare in Iowa as it is in all parts of its range. The writer possesses only a single specimen found under a log in wooded pasture land. Much digging and searching failed to produce any more specimens. This ant is extremely hypogeic.

3. Ponera

KEY TO SPECIES OF PONERA

1. Middle tibial spurs more than one-half the length of hind tibial spurs; erect hairs numerous .....1. *P. coarctata pennsylvanica*
Middle tibial spurs less than one-half the length of hind spurs; erect hairs sparse .....2. *P. trigona opacior*

[3] Although some recent authors are still calling this opening the anus, Emery (1922a) has conclusively shown that the anus or cloacal opening of the Formicinae is between the hypopygium and pygidium, as in every other subfamily of Formicidae.

1. *Ponera coarctata pennsylvanica* Buckley

1866 *P. pennsylvanica* Buckley, Proc. Ent. Soc. Philad., 6:171. ☿
1895 *P. coarctata pennsylvanica* Emery, Zool. Jahrb. Syst., 8:267. ☿ ♀ ♂

Records: Ames, Clinton, Inwood, Muscatine, Oak Grove State Park, Sabula. Also Sioux City (C. N. Ainslie).

This species is the commonest Ponerine in Iowa. It is common near Ames and probably occurs over much of the state. My list of localities could probably be greatly expanded if a more intensive search were made for it. *P. pennsylvanica* is rather hypogeic in habit and nests in small colonies.

2. *Ponera trigona opacior* Forel

1893 *P. trigona* var. *opacior* Forel, Trans. Ent. Soc. Lond., p. 363. ☿ ♀
1895 *P. trigona* var. *opacior* Emery, Zool. Jahrb. Syst., 8:268. ☿ ♀ ♂

Records: Clinton, Glenwood, Little Sioux.

Iowa probably marks the northern limit of the range of this species. Since it was found only on opposite sides of the state, *opacior* has probably managed to creep sporadically northward only along the bluffs of the Mississippi and Missouri rivers.

2. SUBFAMILY DORYLINAE

1. Eciton

KEY TO SPECIES OF ECITON

1. Head and thorax entirely opaque.....1. *E. nigrescens*
   Head and pleurae of prothorax shining .....2. *E. opacithorax*

1. *Eciton* (*Neivamyrmex*) *nigrescens* (Cresson)

1872 *Labidus nigrescens* Cresson, Trans. Amer. Ent. Soc., 4:194. ♂
1894 *Eciton* (*Acamatus*) *schmitti* Emery, Bull. Soc. Ent. Ital., 26:183. ☿
1908 *Eciton* (*Acamatus*) *nigrescens* Wheeler, Bull. Amer. Mus. Nat. Hist., 24:417. ♂
1938 *Eciton* (*Acamatus*) *nigrescens* M. R. Smith, Proc. Ent. Soc. Wash., 40(6):157–160. ☿ ♂
1940 *Eciton* (*Neivamyrmex*) *nigrescens* Borgmeier, Rev. de Ent. Brasil, 11:606.

Records: Little Sioux, Sioux City. Also Sioux City (C. N. Ainslie).

This species can be found in Iowa only along the bluffs of the Missouri River. After rains they may be found marching in long columns. Sioux City is the farthest north that any Doryline ant has ever been taken, but since they appear to closely follow the Missouri River bluffs, it is quite possible that the range of *nigrescens* extends into South Dakota.

2. *Eciton* (*Neivamyrmex*) *opacithorax* Emery

1894 *E.* (*Acamatus*) *californicum opacithorax* Emery, Bull. Soc. Ent. Ital., 26:184. ☿
1900 *E.* (*Acamatus*) *opacithorax* Emery, Mém. Accad. Sci. Bologna, 8(5):23 ☿
1901 *E.* (*Acamatus*) *opacithorax* Wheeler and Long, Amer. Natur., 35:163, 173. ♀ ♂

Record: Glenwood.

This species, found accidentally, was tunneling an inch or two beneath the ground. This is the farthest north this species has been taken. It is rarer than *nigrescens*, but it is quite possible that its range extends as far north.

3. SUBFAMILY MYRMICINAE

KEY TO GENERA OF MYRMICINAE

1. Postpetiole articulated to dorsal surface of gaster, which is flattened dorsally, more convex ventrally, and pointed at the tip .....7. *Crematogaster*
   Postpetiole articulated to anterior end of gaster, which is of a different shape ...2
2. Antennae 6-jointed; the scapes retractible into long scrobes; head cordiform .....11. *Strumigenys*
   Antennae with more than six joints 3
3. Antennae 10-jointed, funicular clubs 2-jointed. .....9. *Solenopsis*
   [one Iowa species, *S. molesta* (Say)]
   Antennae different 4
4. Epinotum with two pairs of spines (anterior pair feeble and dorsally projecting) .....10. *Myrmecina*
   (one Iowa species, *M. graminicola americana* Emery)
   Epinotum different 5
5. Last three joints of the funiculus forming a club as long as or longer than the remainder 6
   Last three joints not as long as the remainder of the funiculus, although the last three joints may form an indistinct club 8
6. Thorax without any trace of teeth or spines .....8. *Monomorium*
   Epinotum with at least feeble spines; integument often strongly sculptured.....7
7. Workers strongly dimorphic, usually without intermediates; scapes of minor workers reaching beyond the head .....5. *Pheidole*
   Workers monomorphic; scapes usually not reaching the hind border of the head .....6. *Leptothorax*
8. Gula with a basket of long hairs .....2. *Pogonomyrmex*
   [one Iowa species, *P. occidentalis* (Cresson)]
   Gula with only normal hairs 9
9. Posterior tibial spurs pectinated; head and thorax strongly rugose...1. *Myrmica*
   Posterior tibial spurs simple 10
10. Small hypogeic species with vestigial eyes and two keels on the clypeus....
    .....3. *Stenamma*
    Medium-sized epigeic species with well-developed eyes and no keels on the clypeus .....4. *Aphaenogaster*

1. Myrmica

KEY TO SPECIES OF MYRMICA

1. Scape without a lobe or lamina at the bend near the base; gaster punctate .....4. *M. punctiventris*
   Scape with a lobe or lamina at the bend 2
2. Lamina of the scape carried partially around the bend and then ventrad along the medial side of the base of the scape; postpetiole convex beneath in profile..
   .....3. *M. schencki emeryana*
   Lamina carried completely around the bend and attached to both sides of the scape distal to the bend; postpetiole straight beneath.....3
3. Lamina of the scape produced into a large spoon-shaped lobe.....
   .....1. *M. sabuleti trullicornis*
   Lamina of the scape not produced into a lobe.....2. *M. sabuleti americana*

1. **Myrmica sabuleti** subsp. **trullicornis** n. subsp.

WORKER. Length about 5.5 mm.

Head, excluding the mandibles, slightly (about one-twentieth) longer

than broad, with moderately convex sides, and feebly convex or slightly excised posterior border. Frontal carinae produced into large lobes projecting dorso-laterally from the head, strongly converging behind. Scape bent at right angles near the base, the bend fitted on the dorsal side with a relatively enormous lobe much larger than in any other previously described North American form except *schencki spatulata* M. R. Smith. When seen from above, this lobe is rather circular in outline and distinctly concave so that it appears much like a ladle. Its very sharp edges are produced along each side of the scape for a short distance.

Thorax with obtuse mesoepinotal impression. Epinotal spines about one-half again as long (about .38 mm. long) as the distance between their bases. In profile the petiole as high as the distance between its ventral tooth and the postpetiole; dorsal surface of petiolar node nearly straight for a short distance before dropping abruptly behind. Postpetiole six-sevenths as long as high, with very convex dorsum in profile, ventral surface nearly straight as in *sabuleti americana* Weber.

Clypeus and mandibles longitudinally striate. Frontal area striato-punctate. Front with about 13 strong longitudinal striae which tend to diverge behind and fuse with the strong reticulate sculpture of the rest of the head. Thorax longitudinally rugose, the rugae larger, and more vermiculate on the pronotum than behind. On the pleurae of the epinotum the rugae converge toward and disappear upon the epinotal spines, about 8–10 rugae taking part in this effect on each side. Dorsa and pleurae of petiole and postpetiole rugose, more irregularly and not so deeply rugose on the dorsa. Gaster smooth and shining.

Pilosity much as in *americana,* the erect hairs moderately abundant, those on the occiput and thorax with blunt tips. Hairs on the scapes oblique, those on the legs subappressed to oblique. Pubescence sparse on all parts.

Color blackish brown; dorsum of the head and gaster darker than the other regions.

FEMALE. Not differing appreciably from the female of *americana* Weber except in having large ladle-shaped lobes on the scapes, somewhat finer sculpture, and darker color.

Described from 34 workers collected April 30, 1941, 4 workers collected August 5, 1939, from woodland colonies near Ames (type locality), and 11 workers and a female from woods near Boone, collected May 3, 1941.

The very similar manner in which the ladle-shaped lobes are attached to the scapes, the almost identical shape of the postpetiole, and many other similarities, show *trullicornis* to be most closely related to *americana* Weber. *M. trullicornis* may be distinguished from *americana* by the large lobe on the scape, which in *americana* is produced only as a small lamina curved around the bend. The sculpture of *trullicornis* seems finer, and the color is darker. *M. trullicornis* also seems to be a woodland form whereas *americana* prefers prairies and open fields. The writer possesses

a series of workers from Sauk Rapids, Minnesota, showing well-marked intergradation between *trullicornis* and *americana*. These forms therefore seem to be no more than subspecifically distinct.

The placement of *trullicornis* and *americana* under *sabuleti* Meinert seems somewhat incongruous as the lamina of the scape is quite differently constructed in this species. Possibly *americana* will prove specifically distinct from *sabuleti*, and *trullicornis* can then be placed as a subspecies of *americana*, and considered as an ecological race of it.

M. sabuleti trullicornis should not be confused with *M. schencki spatulata* M. R. Smith. The frontal carinae are not at all produced into lobes in *spatulata*, the shape and attachment of the large lobe on the scape are quite different, the sculpture is coarser, and the postpetiole is convex beneath.

2. *Myrmica sabuleti americana* Weber

1939 *M. sabuleti americana* Weber, Lloydia, 2:144.

Records: Ames, Boone, Clinton, Keokuk, Jewell, Oak Grove State Park, Granite. Also Sioux City (C. N. Ainslie).

This ant seems common all over Iowa. It prefers to nest in open fields.

3. *Myrmica schencki emeryana* Forel

1914 *M. scabrinodis schencki* var. *emeryana* Forel, Deutsche Ent. Zeitschr., p. 617. ☿ ♀ ♂

Records: Ames, Spirit Lake, Boone, Clinton, Sabula, Inwood.

This ant is fairly common in woodlands in Iowa.

4. *Myrmica punctiventris* Roger

1863 *M. punctiventris* Roger, Berlin Ent. Zeitschr., 7:190. ☿
1886 *M. punctiventris* Mayr, Verh. Zool.-bot. Ges. Wien, 36:450. ☿ ♀
1895 *M. punctiventris* Emery, Zool. Jahrb. Syst., 8:312. ♂

Record: Belle Plaine.

M. punctiventris prefers to live in dense woodlands and is probably much rarer in Iowa than in the eastern states.

2. Pogonomyrmex

1. *Pogonomyrmex* (*Pogonomyrmex*) *occidentalis* (Cresson)

1865 *Myrmica occidentalis* Cresson, Proc. Ent. Soc. Philad., 4:426. ☿ ♀
1882 *Pogonomyrmex occidentalis* McCook, The Honey Ant, etc., p. 123–162. ☿ ♀ ♂

Record: Sioux City (C. N. Ainslie).

This record may be the result of mislabeling. The writer has failed to take this species in Iowa, even along the Missouri River bluffs area. It is, of course, possible that it may sporadically occur in this area.

3. Stenamma

KEY TO SPECIES OF STENAMMA

1. Eyes with approximately 30 facets, head and thorax uniformly opaque .... 1. *S. brevicorne*
   Eyes with about 15 facets; pronotum somewhat shining; mesoepinotal impression deeper ..... 2. *S. brevicorne impressum*

1. *Stenamma brevicorne* (Mayr)

1886 *Aphaenogaster brevicorne* Mayr, Verh. Zool.-bot. Ges. Wien, 36:447. ☿ ♀
1895 *Stenamma brevicorne* Emery, Zool. Jahrb. Syst., 8:298. ☿ ♀ ♂

Records: Ames, Clinton, McGregor, DeWitt. Also Arnolds Park (Judson McQuire); Sioux City (C. N. Ainslie).

This ant is probably common in woodlands over much of the state. It is hypogeic in habit. The winged forms apparently overwinter in the nests as adults or pupae, as they may be found in the nests in early spring.

2. *Stenamma brevicorne impressum* Emery

1895 *S. westwoodi diecki* var. *impressum* Emery, Zool. Jahrb. Syst., 8:301. ☿ ♀
1901 *S. brevicorne diecki* var. *impressum* Forel, Ann. Soc. Ent. Belg., 45:347.

Record: Tama.

As this subspecies is represented by only one specimen, it must be a rare form in Iowa. The specimens which the author previously referred to *impressum* (1941a) belong to the typical *brevicorne*.

4. Aphaenogaster

KEY TO SPECIES OF APHAENOGASTER

1. Scape with a flattened lobe at the base ..... 5. *A. treatae*
   Scape without a lobe at the base 2
2. Basal third of first gastric segment striate ..... 3. *A. mariae*
   First gastric segment not striate 3
3. Epinotal spines longer than the base of the epinotum; few or no hairs on gaster; color deep red ..... 4. *A. tennesseensis*
   Epinotal hairs much shorter than the base of the epinotum; gaster hairy; reddish brown to black 4
4. Reddish brown ..... 1. *A. fulva aquia*
   Deep brown or black ..... 2. *A. fulva picea*

1. *Aphaenogaster* (*Attomyrma*) *fulva aquia* (Buckley)

1867 *Myrmica* (*Monomorium*) *aquia* Buckley, Proc. Ent. Soc. Philad., 6:431. ☿
1895 *Stenamma* (*Aphaenogaster*) *fulva aquia* Emery, Zool. Jahrb. Syst., 8:304. ☿ ♀ ♂
1922 *Aphaenogaster* (*Attomyrma*) *fulva aquia* Emery, Gen. Insec., fasc. 174:57.

Records: Ames, Boone, Holy Cross, Clinton, Dubuque.
Common in all wooded portions of the state.

2. *Aphaenogaster* (*Attomyrma*) *fulva picea* Emery

1895 *Stenamma* (*Aphaenogaster*) *fulva aquia* var. *picea* Emery, Zool. Jahrb. Syst., 8:305. ☿ ♀ ♂

1922 *Aphaenogaster* (*Attomyrma*) *fulva aquia* var. *picea* Emery, Gen. Insec., fasc. 174:57.

Records: Ames, Backbone State Park, Glenwood, Waubonsie State Park, Oak Grove State Park, Clinton.

This variant does not seem very distinct. Perhaps it may be more distinct in the Eastern States but intergrades with *aquia* rather readily in Iowa. Its colonies always seem rather depauperate compared with those of *aquia*.

3. *Aphaenogaster* (*Attomyrma*) *mariae* Forel

1886 *Aph. mariae* Forel, Ann. Soc. Ent. Belg., 30(C.R.):41. ☿

Record: Ames.

The host of this ant is *Aphaenogaster fulva aquia*. The writer on three occasions found *mariae* with *aquia* near Ames. It is much rarer than *tennesseensis*, a very similar and probably closely related species.

4. *Aphaenogaster* (*Attomyrma*) *tennesseensis* (Mayr)

1862 *Atta tenneseensis* Mayr, Verh. Zool.-bot. Ges. Wien, 12:743. ☿
1922 *Aphaenogaster* (*Attomyrma*) *tenneseensis* Emery, Gen. Insec., fasc. 174:60.

Records: Ames, Oak Grove State Park, Rice Lake State Park, Clinton, Belle Plaine, Denison, Boone. Also Sioux City (C. N. Ainslie).

The temporary host of this ant is *A. fulva aquia*, with which it is occasionally found under rocks. When the colonies are fully developed they can be found only in rotting wood.

5. *Aphaenogaster* (*Attomyrma*) *treatae* Forel

1886 *Aph. treatae* Forel, Ann. Soc. Ent. Belg., 30(C.R.):40 ☿ ♀ ♂

Records: Little Sioux, Glenwood, Sioux City, Princeton, DeWitt.

As has been stated in the introduction, this species seems to have a discontinuous distribution in Iowa, occurring only in the extreme eastern and western parts of the state.

5. Pheidole

KEY TO SPECIES OF PHEIDOLE

1. Thorax and gaster with only sparse, clavate hairs ..... 3. *P. sitarches*
   Thorax and gaster with numerous, slender hairs 2
2. Soldiers with occipital lobes of head shining; workers with shining heads .... 1. *P. bicarinata*
   Occipital lobes of soldiers reticulate-rugose; workers with heads punctate and opaque ..... 2. *P. pilifera*

1. *Pheidole* (*Pheidole*) *bicarinata* Mayr

1870 *Ph. bicarinata* Mayr, Verh. Zool.-bot. Ges. Wien, 20:982, 989. ♃

Records: Clinton, Ames, Akron, Burlington, Oak Grove State Park, McGregor, Hinton. Also Sioux City (C. N. Ainslie).

This species is common over most of Iowa. It seems to thrive well in our cities and towns, even though it was originally a member of the prairie fauna. It is less granivorous than *pilifera*.

In the author's preliminary list (1941a) *bicarinata* was misidentified as *P. vinelandica* Forel, a closely related species.

2. *Pheidole* (*Pheidole*) *pilifera* (Roger)

1863 *Leptothorax pilifer* Roger, Berlin Ent. Zeitschr., 7:180. ☿
1886 *Pheidole pennsylvanica* Mayr, Verh. Zool.-bot. Ges. Wien, 36:455. ♃ ☿ ♀ ♂
1895 *Pheidole pilifera* Emery, Zool. Jahrb. Syst., 8:290.

Records: Ames, Bellevue, DeWitt, Princeton.

This ant is numerous in prairie lands. It does not thrive well in our cities and towns, but the writer has occasionally seen it in such situations. *P. pilifera* will accept dead insects if offered, but is largely granivorous.

3. *Pheidole* (*Pheidole*) *sitarches* Wheeler

1908 *Ph. sitarches* Wheeler, Bull. Amer. Mus. Nat. Hist., 24:440. ♃ ☿ ♀

Record: Glenwood.

This species was originally described from Texas and apparently occurs in Iowa only in the extreme southwestern part of the state along the bluffs of the Missouri River.

6. Leptothorax

KEY TO SPECIES OF LEPTOTHORAX

1. Antennae 11-jointed 2
   Antennae 12-jointed 6
2. Thorax with a faint but distinct mesoepinotal constriction (subgenus *Mychothorax*) ..... 6. *L. acervorum canadensis* Provancher
   Thorax without a mesoepinotal constriction 3
3. Epinotal spines very short, dentiform; color black.... 4. *L. fortinodis melanotica*
   Epinotal spines longer 4
4. Head shining; color black..... 3. *L. longispinosus laeviceps*
   Head sculptured; color yellow 5
5. Spines long and curved, their bases approximate..... 1. *L. curvispinosus*
   Spines shorter and straight, their bases farther apart..... 2. *L. ambiguus*
6. Without mesoepinotal constriction; postpetiole much broader than petiole.... ..... 5. *L. tricarinatus*
   With deep mesoepinotal constriction; petiole pedunculate (subgenus *Dichothorax*) ..... 7. *L. pergandei*

1. *Leptothorax* (*Leptothorax*) *curvispinosus* Mayr

1886 *L. curvispinosus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:451, 453. ☿ ♀
1886 *L. curvispinosus* Mayr, Verh. Zool.-bot. Ges Wien, 36:451, 453. ☿ ♀

Records: Ames, Clinton, Tama, Waubonsie State Park, Belle Plaine, Denison, Granite. Also Sioux City (C. N. Ainslie).

A common woodland form. Several colonies have been found nesting in dried hollow stems of plants near Ames.

2. *Leptothorax (Leptothorax) ambiguus* Emery

1895 *L. curvispinosus ambiguus* Emery, Zool. Jahrb. Syst., 8:320. ☿
1940 *L. ambiguus* Wesson and Wesson, Amer. Midl. Nat., 24(1):97.

Records: Boone, Ames.

A much rarer ant than *curvispinosus*. No nests were found. All specimens were either caught in sweeping or with an aspirator as they were crawling on the ground.

3. **Leptothorax (Leptothorax) longispinosus** subsp. **laeviceps** n. subsp.

WORKER. Length, 2.2 mm.

Head oblongate, one-eighth longer than broad, excluding the mandibles. Antennae 11-jointed; scapes nearly reaching the posterior corners of the head. Funicular joints 2–7 broader than long; club one-fifth longer than rest of funiculus. Dorsum of thorax moderately and evenly convex in profile. Epinotal spines long, straight, sharp, somewhat diverging seen from above, projecting backwards and slightly upward, about one-third as long as the distance from bases to neck of pronotum. Petiole and postpetiole much as in *longispinosus* s. str. Petiole a little larger in profile than postpetiole, the anterio-ventral spine very weak, node bluntly subtriangular in profile and not quite as high as in typical *longispinosus*.

Head smooth and shining except for the cheeks, which are striate. Median lobe of clypeus rather indistinctly striate. Thorax striato-punctate, especially on the pleurae; the dorsum feebly shining. The interrugal spaces of the thorax are distinctly wider than in typical *longispinosus*. Petiole and postpetiole punctate.

Erect hairs somewhat clavate, arranged as in typical *longispinosus*. Dark brownish black.

Described from 12 specimens found under a stone on the high Mississippi River bluffs near McGregor, Iowa, June 10, 1940. The typical *longispinosus* apparently does not range as far west as Iowa. *L. laeviceps* may therefore be regarded as a depauperate, geographical race.

L. laeviceps differs from *longispinosus* s. str. in being smaller, and having the sculpturing distinctly less coarse on all parts. The striae on the thorax are farther apart, leaving room for large interrugal punctures. The dorsum of the thorax is a little more convex in profile, and the epinotal spines project a little upward rather than being horizontal. The petiole and postpetiole are not quite as robust. The color of *longispinosus* is often pitch black. The sculpture of the head of *laeviceps* is probably much like that of *L. schmittii* Wheeler, but this species has 12-jointed antennae and much shorter epinotal spines.

4. *Leptothorax (Leptothorax) fortinodis melanotica* Wheeler

1903 *L. fortinodis* var. *melanotica* Wheeler, Proc. Acad. Nat. Sci. Philad., 55:235. ☿ ♀
1940 *L. schaumi* var. *fortinodis* Wesson and Wesson, Amer. Midl. Nat., 24(1):96.

Records: Ames, DeWitt, Clinton.

As Wesson and Wesson have stated (*loc. cit.*) this form may be synonymous with the typical *fortinodis* Mayr, which in turn may be no more than a subspecies of *schaumi* Roger. The author would point out, however, that even though specimens referable to *melanotica* may intergrade indistinguishably with *fortinodis* in the East, *melanotica* may be a valid geographical race in the Middle West, from which region it was described. All the author's specimens are pitch black as described.

5. *Leptothorax (Leptothorax) tricarinatus* Emery

1895 *L. tricarinatus* Emery, Zool. Jahrb. Syst., 8:321. ☿

Records: Inwood, Oak Grove State Park. Also Sioux City (C. N. Ainslie).

This species may be distinguished immediately from all the other species of typical *Leptothorax* in Iowa by its 12-jointed antennae and large postpetiole. *L. tricarinatus* is not related to the members of the subgenus *Dichothorax* which also have 12-jointed antennae.

L. tricarinatus nests in the ground in small colonies.

6. *Leptothorax (Mychothorax) acervorum canadensis* Provancher

1887 *L. canadensis* Provancher, Addit. Faune Canada, Hym., p. 245. ☿ ♀ ♂
1903 *L. acervorum canadensis* Wheeler, Proc. Acad. Nat. Sci. Philad., 55:225. ☿ ♀

Record: Spirit Lake.

This species was found nesting under the bark of a log. *L. acervorum canadensis* is a boreal species, apparently rare in Iowa even in the northern part.

7. *Leptothorax* (*Dichothorax*) pergandei Emery

1895 *L.* (*D.*) *pergandei* Emery, Zool. Jahrb. Syst., 8:318, 323. ☿ ♀ ♂

Records: Boone, Elkader, Glenwood, Bellevue, Dubuque, Sabula.

This ant nests in soil on sunny hillsides. It seems more xerophilous and moves more rapidly than the species of typical *Leptothorax*. It is common nowhere but, nevertheless, cannot be considered very rare in Iowa.

7. Crematogaster

Key to Species of Crematogaster

1. Black in color; thorax opaque (subgenus *Crematogaster* s. str.) 1. C. *lineolata* (Say)
   Mostly yellow; thorax shining (subgenus *Orthocrema*) .....
   .....2. C. *minutissima missouriensis* Emery

1. *Crematogaster (Crematogaster) lineolata* (Say)

1836 *Myrmica lineolata* Say, Boston Jour. Nat. Hist., 1:290. ☿ ♀ ♂
1863 *Crematogaster lineolata* Roger, Verz. Formicid., p. 37.

Records: Ames, Mt. Vernon, Sabula, Keokuk, Muscatine, McGregor, Dubuque, Glenwood.

This species occurs all over the state but does not seem especially common.

2. *Crematogaster (Orthocrema) minutissima missouriensis* Emery

1895 *C. victima missuriensis* Emery, Zool. Jahrb. Syst., 8:288 (in footnote). ☿
1939 *C. (O.) minutissima missouriensis* Creighton, Psyche, 46(4):138.

Records: Little Sioux, Glenwood, Sioux City. Also Sioux City (C. N. Ainslie).

This ant is abundant along the Missouri River bluffs but lacking in other parts of the state. It nests in the ground in small colonies.

8. Monomorium

Key to Species of Monomorium

1. Black; all surfaces shining.....1. *M. minimum*
Yellow; head and thorax finely reticulate-punctate ..... 2. *M. pharaonis*

1. *Monomorium (Monomorium) minimum* (Buckley)

1867 *Myrmica (Monomorium) minima* Buckley, Proc. Ent. Soc. Philad., 6:338. ☿ ♀
1895 *Monomorium minutum* var. *minimum* Emery, Zool. Jahrb. Syst., 8:274. ☿ ♀ ♂
1914 *Monomorium minimum* Wheeler, Jour. New York Ent. Soc., 22:42.

Records: Little Sioux, Inwood, Tama, Ames, Boone. Also Sioux City (C. N. Ainslie).

This minute species usually builds small crater nests in the ground. The writer has once taken it from beneath the bark of a log.

2. *Monomorium (Monomorium) pharaonis* (Linné)

1758 *Formica pharaonis* Linné, Syst. Nat., ed. 10, 1:580.
1862 *Monomorium pharaonis* Mayr, Verh. Zool.-bot. Ges Wien, 12:752.

Record: Ames.

This species does not live out-of-doors in these latitudes. It is occasionally found in buildings and houses, where it apparently nests in the walls.

9. Solenopsis

1. *Solenopsis (Diplorhoptrum) molesta* (Say)

1836 *Myrmica molesta* Say, Boston, Jour. Nat. Hist., 1:293. ♀
1895 *Solenopsis molesta* Emery, Zool. Jahrb. Syst., 8:277. ☿ ♀ ♂

Records: Ames, Sioux City, Boone, Marshalltown, Inwood, Tama, Belle Plaine. Also Sioux City (C. N. Ainslie).

Probably very abundant over the entire state.

10. Myrmecina

1. *Myrmecina graminicola americana* Emery

1895 *M. latreillei americana* Emery, Zool. Jahrb. Syst., 8:271. ☿
1922 *M. graminicola americana* Emery, Gen. Insec., fasc. 174:232.

Records: DeWitt, Clinton, Ames, Boone.

Winged males were found in a nest in late August. This ant is strictly hypogeic.

M. americana differs from the typical European *graminicola* rather distinctly. The scapes of *americana* are not flattened and broad at the base but are circular in cross section; the clypeal teeth are less distinct and the median clypeal carina nearly absent, the head is slightly broader than long rather than a little longer than broad, and is also distinctly excised behind. The thorax is a little broader in proportion to its length, and the anterior epinotal spines are better developed. *M. americana* may thus deserve to rank as a good species.

11. Strumigenys

Key to Species of Strumigenys

1. Visible portion of mandibles one-third as long as the head, a basal tooth visible just before the clypeus ..... 1. *S. pergandei*
   Visible portion of mandibles one-fifth as long as the head, no basal tooth visible ..... 2. *S. pulchella*

1. *Strumigenys (Cephaloxys) pergandei* Emery

1895 *S. pergandei* Emery, Zool. Jahrb. Syst., 8:326. ☿ ♀ ♂
1931 *S. (C.) pergandei* M. R. Smith, Ann. Ent. Soc. Amer., 24(4):698.

Records: Boone, Holy Cross, Bellevue.

A rare species in Iowa. It is usually found near the nests of other ants.

2. *Strumigenys (Cephaloxys) pulchella* Emery

1895 *S. pulchella* Emery, Zool. Jahrb. Syst., 8:327. ☿
1931 *S. (C.) pulchella* M. R. Smith, Ann. Ent. Soc. Amer., 24(4):702. ☿

Record: Ames.

This species is either extremely rare or extremely hypogeic in Iowa. In the spring, single workers can rarely be found under rocks in damp soil.

4. SUBFAMILY DOLICHODERINAE

Key to Genera of Dolichoderinae

1. Epinotum with a conical point ..... 2. *Dorymyrmex*
   Epinotum without a conical point 2

2. Petiolar scale very small and strongly inclined forward, not distinct..3. *Tapinoma* [one Iowa species, *T. sessile* (Say)]
Petiolar scale distinct, more erect, sharply pointed above.....1. *Iridomyrmex* [one Iowa species, *I. pruinosum analis* (Ern. André)]

1. Iridomyrmex

1. *Iridomyrmex pruinosum analis* (Ern. André)

1893 *Tapinóma anale* Ern. André, *Rev. Entom.*, p. 148. ☿
1895 *Tapinoma pruinosum* var. *anale* Emery, Zool. Jahrb. Syst., 8:333.
1912 *Iridomyrmex analis* Emery, Gen. Insec., fasc. 137:26.

Records: Glenwood, Inwood, Oak Grove State Park, Little Sioux. Also Sioux City (C. N. Ainslie).

This species is common along the Missouri River bluffs and also in prairie remnants in the area drained by the Missouri River system. It does not occur in central or eastern Iowa.

2. Dorymyrmex

Key to Species of Dorymyrmex

1. Head and thorax brown, lighter than gaster.....1. *D. pyramicus*
Color uniformly black .....2. D. *pyramicus niger*

1. *Dorymyrmex pyramicus* (Roger)

1863 *Prenolepis pyramica* Roger, Berl. Ent. Zeitschr., 7:160. ☿
1886 *Dorymyrmex pyramicus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:365, 433. ☿♀
1895 *Dorymyrmex pyramicus* Emery, Zool. Jahrb. Syst., 8:331. ♂

Records: Sioux City, Little Sioux, Oak Grove State Park, Inwood. Also Sioux City (C. N. Ainslie).

The distribution of this species in Iowa is the same as that of *Iridomyrmex analis*. Some of the author's specimens seem somewhat transitional to *D. pyramicus niger*.

2. *Dorymyrmex pyramicus niger* Pergande

1895 *D. pyramicus* var. *niger* Pergande, Proc. Calif. Acad. Sci., 5(2):871.

Record: Ames:

Besides the darker, uniform color of the specimens the writer has referred to *niger*, there are also these differences between them and typical *pyramicus*. The head of *niger* is a little more elongate, the scapes slightly longer, the second funicular joint shorter, the mesoepinotal suture not deeply impressed, and the petiole smaller and blunter.

This form nests only in virgin prairie or open fields. On a virgin prairie remnant near Ames it was especially abundant, more abundant than any other ant. It seems probable that this form was a dominant species in the original prairie fauna of Iowa before cultivation extinguished it. *D. niger* can be found occasionally in pasture lands but does

not thrive in such situations. Recently the virgin prairie remnant mentioned above has been overgrazed and the prairie flora has disappeared. The writer has noticed a decrease in the number of *D. niger* there, apparently correlated with this change.

3. Tapinoma

1. *Tapinoma sessile* (Say)

1836 *Formica sessilis* Say, Boston Jour. Nat. Hist., 1:287. ♀
1863 *Tapinoma boreale* Roger, Berl. Ent. Zeitschr., 7:165. ☿♀
1895 *Tapinoma sessile* Emery, Zool. Jahrb. Syst., 8:332. ♀♂

Records: Ames, Clinton, Burlington, Tama, Inwood. Also Sioux City (C. N. Ainslie).

This species is so common in woodlands near Ames that it undoubtedly is common over most of the state. The writer has not seen it on the Missouri River bluffs, where *Iridomyrmex analis* apparently takes its place.

5. SUBFAMILY FORMICINAE

Key to Genera of Formicinae

1. Antennae 9-jointed ..... 1. *Brachymyrmex*
   (one Iowa species, *B. depilis* Emery)
   Antennae with 12 joints 2
2. Metathoracic spiracles on the sides of the thorax; insertions of the antennae relatively distant from the clypeus ..... 2. *Camponotus*
   Metathoracic spiracles on the dorsum near the mid-line; insertions of the antennae close behind the clypeus 3
3. Clypeal fossae separate from the antennal fossae; petiolar scale inclined forward.. 4
   Clypeal fossae confluent with the antennal fossae; petiolar scale erect 5
4. Mesothorax strongly constricted ..... 4. *Prenolepis*
   [one Iowa species, *P. imparis* (Say)]
   Mesonotum not constricted; an impressed suture just behind metathoracic spiracles ..... 3. *Paratrechina*
5. Second and third funicular joints together at least slightly shorter than the first; second to fifth joints shorter than the succeeding joints; ocelli usually absent ..... 5. *Lasius*
   Second and third funicular joints together at least slightly longer than the the first; second to fifth joints at least as long as succeeding joints; ocelli present 6
6. Mandibles falcate, their teeth vestigial ..... 7. *Polyergus*
   Mandibles normal in shape, with broad dentate apical borders..... 6. *Formica*

1. Brachymyrmex

1. *Brachymyrmex* (*Brachymyrmex*) *depilis* Emery

1893 *B. heeri depilis* Emery, Zool. Jahrb. Syst., 7:635. ☿♀♂
1923 *B.* (*B.*) *depilis* Santschi, An. Mus. Hist. Nat. Buenos Aires, 31:653, 663. ☿

Records: McGregor, Oak Grove State Park.

Besides the two listed localities I have also seen fragments of these

ants in excreta of shrews from the vicinity of Ames. It is probably a common species but because of its extremely small size and hypogeic habits, not often found.

2. Camponotus

Key to Species of Camponotus

1. Clypeus entire anteriorly or only very broadly notched, large species up to 13 mm.....2
   Clypeus notched; small species up to 7 mm. (subgenus *Myrmentoma*).....5
2. Head of major worker longer than wide; body somewhat shining.....3
   Head of major worker wider than long; body more opaque 4
3. Head black, thorax and gaster dark brown .....4. *C. castaneus americanus*
   Head dark brown; thorax and gaster tan, scarcely infuscated.....3. *C. castaneus*
4. Thorax black, gaster with long pubescence .....1. *C. herculeanus pennsylvanicus*
   Thorax red, gaster with only short pubescence..2. *C. herculeanus novaeboracensis*
5. Cheeks and clypeus with elongate, piligerous foveolae 6
   Cheeks and clypeus without elongate, piligerous foveolae 7
6. Head and thorax largely black or dark brown.....8. *C. caryae subbarbatus*
   Head and thorax red .....7. *C. caryae discolor*
7. Head and thorax largely black .....5. *C. caryae nearcticus*
   Head and thorax red .....6. *C. caryae rasilis*

1. *Camponotus* (*Camponotus*) *herculeanus pennsylvanicus* (Degeer)

1773 *Formica pennsylvanica* Degeer, Mém. Hist. Insect., 3:603. ☿♀♂
1879 *Camponotus herculeanus pennsylvanicus* Forel, Bull. Soc. Vaud. Sci. Nat., 16:57.

Records: Ames, Princeton, Little Sioux, Glenwood, Sioux City, Ruthven. Also Sioux City (C. N. Ainslie).

This species always lives in galleries which it excavates in solid or rotten wood. It occasionally nests in the beams of frame houses, weakening them considerably. Incipient colonies consisting of a female and several minor workers can often be found just under the bark of logs. The above list of localities could be considerably lengthened as *pennsylvanicus* is common in every woodland.

2. *Camponotus* (*Camponotus*) *herculeanus novaeboracensis* (Fitch)

1854 *Formica novaeboracensis* Fitch, Trans. New York State Agric. Soc., 14:52. ☿
1910 *Camponotus herculeanus ligniperda* var. *noveboracensis* Wheeler, Ann. New York Acad. Sc., 20:340. ☿ ♀ ♂
1925 *Camponotus (C.) herculeanus* var. *novaeboracensis* Emery, Gen. Insec., fasc. 183:72.

Records: Ames, Estherville, Holy Cross, Spirit Lake, Backbone State Park, Rice Lake State Park. Also Indianola (D. T. Jones).

This ant nests in wood as *pennsylvanicus* does. It appears to have a more boreal distribution than *pennsylvanicus* and does not occur in the southern part of Iowa. *C. novaeboracensis* and *pennsylvanicus* sometimes occur in the same locality, apparently without intergradation. It seems, therefore, that they could be considered specifically, rather than only subspecifically distinct.

3. *Camponotus* (*Camponotus*) *castaneus* (Latreille)

1802 *Formica castanea* Latreille, fourmis, p. 118. ☿ ♀ ♂
1886 *Camponotus castaneus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:420.

Record: Burlington.

This southern species seems to reach its northern limit in southeastern Iowa. The colony the writer found was nesting under a flat rock in woodland.

4. *Camponotus* (*Camponotus*) *castaneus americanus* Mayr

1862. *C. americanus* Mayr, Verh. Zool.-bot. Ges. Wien, 12:661. ☿ ♀
1893 *C. castaneus americanus* Emery, Zool. Jahrb. Syst., 7:674.

Records: Ames, Clinton, Backbone State Park, Inwood.

This ant nests in the ground, never in wood. It prefers woodlands, however. Winged males and females were taken in nests in early April and May, so evidently these casts overwinter as adults. The winged casts of *herculeanus pennsylvanicus* often overwinter as adults, also.

5. *Camponotus* (*Myrmentoma*) *caryae nearcticus Emery*

1893 *C. marginatus var. nearcticus* Emery, Zool. Jahrb. Syst., 7:675. ☿ ♀
1910 *C. fallax* var. *nearcticus* Wheeler, Jour. New York Acad. Sci., 18:222. ☿ ♀ ♂
1917 *C. (Camponotus) caryae* Wheeler, Psyche, 24:27.

Records: Ames, Tama, Holy Cross, Clinton.

This ant nests in the dead branches of hickory and oak trees. Specimens are not often taken, but it is probably a fairly common woodland form in Iowa.

6. *Camponotus* (*Myrmentoma*) *caryae rasilis* Wheeler

1910 *C. fallax rasilis* Wheeler, Jour. New York Ent. Soc., 18:227. ☿ ♀ ♂
1917 *C. caryae rasilis* Wheeler, Psyche, 24:28.

Record: Sioux City (C. N. Ainslie).

This is another of the southern forms which appear to have extended their range northward along the Missouri River bluffs. The Iowa specimens of *rasilis* collected by Ainslie are all much smaller than the typical *rasilis* of the southern states.

7. *Camponotus* (*Myrmentoma*) *caryae discolor* (Buckley)

1866 *Formica discolor* Buckley, Proc. Ent. Soc. Philad., 6:166. ☿ ♀
1893 *Camponotus marginatus discolor* Emery, Zool. Jahrb. Syst., 7:277. ☿ ♀ ♂
1917 *Camponotus caryae discolor* Wheeler, Psyche, 24:28.

Records: Ames, Boone.

This subspecies apparently has the same nesting habits as *nearcticus*. It is rarer in Iowa than *nearcticus*.

8. *Camponotus* (*Myrmentoma*) *caryae subbarbatus* Emery

1893 *C. marginatus subbarbatus* Emery, Zool. Jahrb. Syst., 7:676: ☿ ♀ ♂
1917 *C. caryae subbarbatus* Wheeler, Psyche, 24:28.

Records: Ames, Boone.

The two colonies of this rare ant that the writer has found have been under or in rotting wood in the ground. It may thus prove to have different nesting habits than the tree-dwelling *nearcticus* and *discolor*.

Several forms of *caryae* other than the four above were included in the writer's preliminary list. These were based on old, faded or otherwise poor material reposing in the Iowa State College collection. The author has decided that their identifications are too doubtful to be included in the present paper.

3. Paratrechina

Key to Species of Paratrechina

1. Scapes with erect hairs; yellow .....1. *P. arenivaga*
   Scapes without erect hairs; black .....2. *P. parvula*

1. *Paratrechina* (*Nylanderia*) *arenivaga* (Wheeler)

1905 *Prenolepis arenivaga* Wheeler, Bull. Amer. Mus. Nat. Hist., 21:391. ☿ ♂
1925 *Paratrechina (N.) arenivaga* Emery, Gen. Insec., fasc. 183:221.

Records: Sioux City, Blencoe, Little Sioux.

A rather common member of the Missouri River bluff fauna but not found in any other part of the state. It builds small crater nests in the loess soil of these bluffs.

2. *Paratrechina* (*Nylanderia*) *parvula* (Mayr)

1870 *Prenolepis parvula* Mayr. Verh. Zool.-bot. Ges. Wien, 20:948. ☿ ♂ ♀
1925 *Paratrechina (N.) parvula* Emery, Gen. Insec., fasc. 183:222.

Records: Ames, Clinton, Inwood, Dubuque, DeWitt.

This species is fairly common in Iowa. It usually nests under rocks in sunny places. The sexual phases apparently overwinter in the nests since they may be found in the nests in early spring.

4. Prenolepis

1. *Prenolepis imparis* (Say)

1836 *Formica imparis* Say, Boston Jour. Nat. Hist., 1:287. ♀ ♂
1886 *Prenolepis imparis* Mayr, Verh. Zool.-bot. Ges. Wien, 36:431.

Records: Ames, Backbone State Park, Clinton.

The sexual casts of *P. imparis* overwinter in the nest and fly in the first warm days of spring. This ant is common in woodlands and also in our cities and towns. It rarely appears above ground except in cool, damp weather.

5. Lasius

Key to Species of Lasius

1. Maxillary palpi 6-jointed 2
   Maxillary palpi 3-jointed (subgenus *Acanthomyops*) 7
2. Last three joints of maxillary palpi subequal in length; eyes large 3
   Last two joints shorter than the fourth joint; eyes small 4
3. Erect hairs present on the scapes ..... 1. *L. niger neoniger*
   Erect hairs lacking on the scapes ..... 2. *L. niger americanus*
4. Scapes not reaching the posterior corners of the head ..... 3. *L. brevicornis*
   Scapes surpassing the posterior corners of the head 5
5. Scapes slightly surpassing the posterior corners of the head; last joint of maxillary palpi as long as the preceding joint; light yellow in color ..... 4. *L. flavus nearcticus*
   Scapes distinctly surpassing posterior corners; last joint of maxillary palpi shorter than preceding joint; color darker (subgenus *Chthonolasius*) 6
6. No or very few erect hairs on gula or legs; gastric pubescence sparse revealing the shining surface ..... 6. *L. umbratus epinotalis*
   Erect hairs present on gula and legs; gastric pubescence dense ..... 5. *L. umbratus aphidicola*
7. Hairs plumose at the distal ends ..... 10. *L. plumopilosus*
   Hairs simple or only feebly barbellate 8
8. Petiole blunt; erect hairs numerous on all femora ..... 9. *L. latipes*
   Petiole sharper and notched above; erect hairs not present on all femora 9
9. Scapes surpassing posterior corners of the head; penultimate joints of funiculi longer than broad ..... 8. *L. interjectus*
   Scapes not or scarcely surpassing posterior corners of the head; penultimate joints slightly broader than long ..... 7. *L. claviger*

1. *Lasius* (*Lasius*) *niger neoniger* Emery

1893 *L. niger* var. *neoniger* Emery, Zool. Jahrb. Syst., 7:639. ☿ ♀ ♂

Records: Ames, Marshalltown, Princeton, Spirit Lake.

This species could probably be found in every square mile in Iowa except along the Missouri River bluffs. It may be our commonest species.

2. *Lasius* (*Lasius*) *niger americanus* Emery

1893 *L. niger* var. *americanus* Emery, Zool. Jahrb. Syst., 7:639. ☿ ♀ ♂
1917 *L. niger alienus* var. *americanus* Wheeler, Proc. Amer. Acad. Art. Sci. Boston, 52:525.

Records: Ames, Clinton.

The paucity of records is due to neglect in collecting the species. In all probability it is at least the second commonest ant in Iowa. It does not thrive well in our cities and towns as *neoniger* does.

Lasius (*Lasius*) *brevicornis* Emery

1893 *L. brevicornis* Emery, Zool. Jahrb. Syst., 7:639. ☿ ♀ ♂

Records: McGregor, Sabula.

This species seems rare in Iowa although undoubtedly many more collections could be made in the northeastern part of the state. It does not occur near Ames.

4. *Lasius (Lasius) flavus nearcticus* Wheeler

1906 *L. flavus nearcticus* Wheeler, Psyche, 13:38.

Records: Ames, Belle Plaine, Spirit Lake.

Apparently rather rare in Iowa. It is found in woodlands under rocks or logs in moist soil. The color of this species is usually given as very light yellow with the gaster whitish. In the writer's opinion the whiteness of the gaster is caused by fading in alcohol. Although somewhat lighter than *umbratus aphidicola*, the true color of *nearcticus* is as dark as that of *brevicornis*.

5. *Lasius (Chthonolasius) umbratus aphidicola* (Walsh)

1862 *Formica aphidicola* Walsh, Proc. Ent. Soc: Philad., 1:310. ☿ ♂
1893 *Lasius umbratus mixtus* var. *aphidicola* Emery, Zool. Jahrb. Syst., 7:640. ☿ ♀ ♂

Records: Ames, Tama, Rice Lake State Park, Sabula, Belle Plaine, Clinton, Marshalltown.

The writer has found a female of *aphidicola* with a depauperate colony of *flavus nearcticus*. This seems to indicate that *nearcticus* is the host or at least an alternate host of *aphidicola*.

6. **Lasius (Chthonolasius) umbratus** subsp. **epinotalis** n. subsp.

WORKER. Length, 3.5 mm.

Head a little longer than broad, with straight posterior border and moderately convex sides. Mandibles 8–9-toothed. Scapes extending beyond posterior corners of head by about one-fourth of their length. Penultimate joints of funiculi a little longer than broad. Eyes with approximately 65 facets. Epinotum usually rounded and without a distinct angle between the base and declivity, the declivity not greatly longer than the base. Petiole cuneate in profile, ordinarily sharp and excised above. Legs rather elongate.

All surfaces shining, especially the gaster. Erect hairs somewhat thicker and longer than on *aphidicola*. Hairs on head and thorax rather long and flexuous, those on the gaster shorter, straight, and numerous. No or very few hairs on gula and legs. Pubescence moderately dense on head and thorax but not concealing the shining surface; rather sparse on the gaster.

Head and thorax sordid yellow; gaster sometimes infuscated.

Described from twenty-six specimens taken under a rock in wooded pasture land near Bellevue, Iowa, June 17, 1941.

The rounded epinotum of this subspecies is very suggestive of the species of the subgenus *Acanthomyops*. All other species of the subgenera *Lasius* s. str. and *Chthonolasius* known to the writer have a more angular epinotum with the declivity usually much longer than the base.

L. epinotalis appears closely related to *aphidicola*, but the longer, more slender antennae, less angular epinotum, smaller eyes, sparser pub-

escence, longer, less fine, rather flexuous erect hairs, which are lacking on gula and legs, and smaller size show it to be distinct.

L. umbratus speculiventris may be easily distinguished from *epinotalis* by the numerous erect hairs on scapes and legs, and lack of pubescence on the gaster. *L. umbratus subumbratus* is perhaps most closely related to *epinotalis* but differs in its larger size, more angular epinotum, somewhat more numerous hairs and pubescence, and in having hairs on the gula. The antennae of *subumbratus* are a little less slender, the eyes a little smaller, and the promesonotum a little more convex, also.

In the author's unpublished thesis this subspecies is described under the manuscript name *L. lucidiventris*.

7. *Lasius* (*Acanthomyops*) *claviger* (Roger)

1862 *Formica clavigera* Roger, Berl. Ent. Zeitschr., 6:241. ♀
1870 *Lasius (A.) claviger* Mayr, Verh. Zool.-bot. Ges. Wien, 20:950. ☿ ♀ ♂

Records: *Ames, Burlington, Muscatine, Bellevue, Sabula, Boone, Belle Plaine, Inwood, Backbone State Park, Marshalltown.

This species is common in woodlands all over the Mississippi River drainage area. Very probably it is parasitic on *Lasius niger neoniger*. Wedding flights take place in late August, September, and even October. Females can sometimes be found in early spring under logs and rocks. These are always without eggs or larvae and probably are females which failed to find a suitable host colony after their wedding flight the previous fall.

8. *Lasius* (*Acanthomyops*) *interjectus* Mayr

1866 *L. (A.) interjectus* Mayr, Verh. Zool.-bot. Ges. Wien, 16:888. ♀
1886 *L. (A.) interjectus* Mayr, Verh. Zool.-bot. Ges. Wien, 36:430.. ☿ ♀ ♂

Records: Ames, Boone, Clinton. Also Sioux City (C. N. Ainslie); Des Moines (collector ?).

This species has been previously reported (Buren, 1941a) as undertaking wedding flights in warm basements in midwinter. Females taken in similar circumstances in Des Moines have been sent to the Department of Zoology and Entomology, Iowa State College. The normal wedding flight takes place in July or early August.

9. *Lasius* (*Acanthomyops*) *latipes* (Walsh)

1862 *Formica latipes* Walsh, Proc. Ent. Soc. Philad., 1:311. ☿ ♀ ♂
1866 *Lasius (A.) latipes* Mayr. Verh. Zool.-bot. Ges. Wien, 16:889.
1903 *Lasius (A.) latipes* Wheeler and McClendon, Biol. Bull., 4:149–155. Female α female β.

Records: Ames, Clinton, Spirit Lake.

This species seems rather rare in Iowa. At least the writer has had poor luck in finding it. The wedding flights take place in August, sometimes on the same day as its probable host, *Lasius niger neoniger*.

10. *Lasius (Acanthomyops) plumopilosus* Buren

1941 *L. (A.) plumopilosus* Buren, Iowa State Coll. Jour. Sci., 15(3):231–235. ☿ ♀ ♂

Type locality: Backbone State Park.

This species may prove to be a temporary social parasite of *L. (A.) claviger*, which would make it one of the rare social hyperparasites. Since the publication of the original description the writer has failed to find this species in any other place except on the hillside where it was first found. There appear to be two or three nests of *plumopilosus* and six or more nests of *claviger* on this hillside.

6. Formica

Key to Species of Formica

1. Second and third funicular joints together little longer than the first, the third never longer than the penultimate; small shining species (subgenus *Proformica*) 2
   Third funicular joint longer or as long as the penultimate, second and third joints together usually distinctly longer than the first; mostly medium to large-sized species; often opaque or the colors red and black 5
2. Scapes with erect hairs ..... 26. *F. neogagates vetula*
   Scapes without erect hairs 3
3. Gaster yellow or tan like the head and thorax ..... 28. *F. neogagates morbida*
   Gaster black or very dark brown 4
4. Whole body black or very dark brown ..... 25. *F. neogagates*
   Thorax lighter than the head and gaster ..... 27. *F. neogagates vinculans*
5. Median joints of funiculi 1½ times or more as long as broad; head and thorax long and slender (subgenus *Neoformica*) 6
   Median joints of funiculi less than 1½ times as long as broad; head and thorax usually more robust (subgenus *Formica* s. str.) 9
6. Erect hairs present on gula and petiole; hairs on gaster slender 7
   Erect hairs absent on gula and petiole; hairs on gaster shorter and blunter 8
7. Hairs on gula and petiole conspicuous; pubescence on gaster longer and denser ..... 30. *F. pallidefulva dolosa*
   Hairs often lacking on gula or petiole; pubescence on gaster shorter and sparser; color usually darker ..... 29. *F. pallidefulva incerta*
8. Head and thorax brown or reddish ..... 31. *F. pallidefulva nitidiventris*
   Head and thorax black or very dark brown ..... 32. *F. pallidefulva fuscata*
9. Clypeus with an anterior median notch (*sanguinea* group) 10
   Clypeus unnotched 13
10. Few or no hairs on dorsal surfaces of head and thorax 11
    Erect hairs present on upper surfaces of head and thorax 12
11. Dorsal surfaces of head infuscated, or the head at least darker than the thorax; both dark red ..... 21. *F. sanguinea aserva*
    Head not darker than the thorax; both lighter red ..... 24. *F. sanguinea subnuda*
12. Gaster brown ..... 23. *F. sanguinea subintegra*
    Gaster black ..... 22. *F. sanguinea rubicunda*
13. Ground color of head and thorax red, although sometimes heavily infuscated; frontal area smooth and shining 14
    Ground color of head and thorax black or at least not red; frontal area pubescent and rather opaque; hairs on gaster blunt (*fusca* group) 28
14. Head deeply excised behind (*exsecta* group) 27
    Head at most feebly excised behind 15
15. Petiole blunt, rather truncate or excised above 16
    Upper border of petiole convexly or angularly produced, although sometimes with a notch in the middle 17
16. Eyes hairy ..... 13. *F. reflexa*
    Eyes hairless ..... 12. *F. dakotensis montigena*

17. Erect hairs and pubescence nearly absent; gaster strongly shining..10. *F. fossaceps*
Erect hairs on pubescence more numerous; integument more opaque.....18
18. Clypeal fossae deep; gaster rather sparsely pubescent, the surface not concealed.19
Clypeal fossae shallow; gaster often densely pubescent.....20
19. Smaller workers infuscated, majors and medium-sized workers with at least the scale of the petiole infuscated .....9. *F. rufa clivia*
Smaller workers hardly darker than the majors, these with the petiole clear red .....8. *F. rufa obscuriventris*
20. Erect hairs absent from dorsal surfaces of head and gaster.....11. *F. prociliata*
Erect hairs present on the dorsa of head and gaster 21
21. Eyes hairy, erect hairs numerous 22
Eyes hairless, erect hairs moderately abundant or sparse 24
22. Erect hairs present on cheeks, oblique hairs on scapes.....14. *F. knighti*
No erect hairs on cheeks, no hair other than the pubescence on scapes; large robust forms 23
23. Pubescence dense on gaster, concealing the surface.....6. *F. rufa obscuripes*
Pubescence scarce on gaster .....7. *F. rufa melanotica*
24. Gaster rather shining, sparsely pubescent.....17. *F. nepticula*
Gaster opaque, densely pubescent 25
25. Hairs apparently clavate; cheeks densely pubescent.....26
Hairs slender; cheeks sparsely pubescent .....18. *F. difficilis*
26. Hairs numerous, present on occipital corners of head....15. *F. microgyna spatulata*
Hairs sparse, not present on occipital corners.....16. *F. indianensis*
27. Front and vertex of head heavily infuscated; pronotum with numerous erect hairs .....20. *F. ulkei*
Head not or scarcely infuscated; pronotum with no or very few hairs.....
.....19. *F. exsectoides*
28. Long erect hairs present on the gula.....5. *F. cinerea neocinerea*
Gula without erect hairs 29
29. Thorax yellowish .....4. *F. fusca neoclara*
Thorax black 30
30. Pubescence long and dense on all parts, giving a silvery appearance.....
.....3. *F. fusca argentea*
Pubescence shorter or less dense; body without a silvery appearance.....31
31. Pubescence dense on the gaster .....1. *F. fusca subsericea*
Pubescence on gaster rather sparse; body more shining..2. *F. fusca subaenescens*

1. *Formica (Formica) fusca subsericea* Say

1836 *F. subsericea* Say, Boston Jour. Nat. Hist., 1:289. ☿ ♀
1913 *F. (Formica) fusca* var. *subsericea* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:398, 499. ☿ ♀ ♂
1925 *F. (Serviformica) fusca subsericea* Emery, Gen. Insec., Fasc. 183:248.

Records: Ames, Clinton, Jewell, Spirit Lake, Backbone State Park, Carroll, Mt. Vernon, Oak Grove State Park. Also Sioux City (C. N. Ainslie).

This ant is common in all woodlands and in our cities and towns. It is the host of a number of parasitic *Formica*.

2. *Formica (Formica) fusca subaenescens* Emery

1893 *F. fusca* var. *subaenescens* Emery, Zool. Jahrb. Syst., 7:659. ☿
1913 *F. (F.) fusca fusca* var. *subaenescens* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:399, 504. ☿ ♀ ♂

Record: Backbone State Park.

This variant is an inhabitant of deep woods where it nests in damp soil under rocks and logs. Apparently it is rare or absent in central Iowa where woodlands are rather scattered and usually somewhat open. *F.*

subaenescens may be the normal host of *Polyergus rufescens bicolor*, as will be shown in the discussion of the latter.

3. *Formica* (*Formica*) *fusca argentea* Wheeler

1902 *F. fusca* var. *argentata* Wheeler, Amer. Nat., 36:952 (in footnote). ☿ (nom. praeocc.)
1912 *F. fusca* var. *argentea* Wheeler, Psyche, 19:90. (nom. nov.)
1913. *F. (Formica) fusca fusca* var. *argentea* Wheeler, Bull. Mus. Comp. Zool., Cambridge, 53:398, 501. ☿ ♀ ♂
53:398, 501. ☿ ♀ ♂
1925 *F. (Serviformica) fusca subsericea* var. *argentea* Emery, Gen. Insec., fasc. 183:248.

Record: Stanhope (from prairie) (G. O. Hendrickson).

This species was probably a member of the original prairie fauna which has now been displaced in a large part by cultivation.

4. *Formica* (*Formica*) *fusca neoclara* Emery

1893 *F. fusca* var. *neoclara* Emery, Zool. Jahrb. Syst., 7:661: ☿
1913 *F. (Formica) fusca fusca* var. *neoclara* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:398, 509. ☿ ♀ ♂
1925 *F. (Serviformica) fusca subsericea* var. *neoclara* Emery, Gen. Insec., fasc. 183:248.

Record: Sioux City (C. N. Ainslie).

The validity of this record is somewhat doubtful as this variant is usually found only in the foothills of the Rocky Mountains. The writer suspects the specimens purported to be from Iowa may have been mislabeled.

5. *Formica* (*Formica*) *cinerea neocinerea* Wheeler

1902 *F. cinerea* Wheeler, Amer. Nat., 36:947.
1910 *F. cinerea* var. *neocinerea* Wheeler, Ants, p. 571. ☿
1913 *F. (Formica) cinerea cinerea* var. *neocinerea* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:399, 524. ☿ ♀ ♂
1925 *F. (Serviformica) cinerea* var. *neocinerea* Emery, Gen. Insec., fasc. 183:246.

Records: Jewell, Ames, Spirit Lake.

This ant prefers to nest in the tops of boggy hummocks in pasture land, and probably could be found in any part of the state where such hummocks are present. It is more aggressive than the forms of *fusca*.

6. *Formica* (*Formica*) *rufa obscuripes* Forel

1886 *F. rufa st. obscuripes* Forel, Ann. Soc. Ent. Belg., 30 (C.R.):29. ☿
1913 *F. (F.) rufa aggerans* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 430. ☿ ♀ ♂
1940 *F. rufa obscuripes* Creighton, Amer. Mus. Nov., 1055:1, 7.

Records: Oak Grove State Park, Inwood, McGregor, Spencer. Also Ruthven (J. B. Low); Thompson (T. S. Baskett); Ocheyedan, Stanhope, Thompson, Westfield (G. O. Hendrickson).

This ant is often called the "thatching ant" because of the large

mound nest composed of twigs and other plant debris which these ants construct. All specimens from Iowa show more melanism, even in the largest workers, than is common in specimens of *obscuripes* from the Great Plains. Thus they may be considered transitional to the following variant, *melanotica.*

7. *Formica (Formica) rufa melanotica* Emery

1893 *F. rufa obscuriventris* var. *melanotica* Emery, Zool. Jahrb. Syst., 7:644, 650. ☿
1913 *F. (F.) aggerans* var. *melanotica* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 432. ☿ ♀ ♂
1940 *F. (F.) rufa melanotica* Creighton, Amer. Mus. Nov., 1055:1, 7.

Record: Denison.

The several nests of this variant near Denison were found in pasture land densely covered by scrub oaks so that all nests were shaded. This is in contradistinction to the nests of the form the writer has referred to *obscuripes,* whose nests were chiefly in virgin prairie, or at least exposed to the sun.

8. *Formica (Formica) rufa obscuriventris* Mayr

1870 *F. rufa obscuriventris* Mayr, Verh. Zool.-bot. Ges. Wien, 20:951. ☿
1913 *F. (Formica) rufa obscuriventris* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:392, 394, 445. ☿ ♀ ♂

Records: Backbone State Park, Dubuque, Muscatine, Mt. Vernon.

This variant constructs its nests in old, dry, rotted stumps or logs, filling up the cavities with plant debris. It is very fierce and aggressive in the defense of its nest, like *obscuripes* and *melanotica.* In macroscopic aspect, color, size, aggressiveness, nesting habits, etc., this ant is almost identical with *F. sanguinea aserva.*

9. *Formica (Formica) rufa clivia* Creighton

1917 *F. (F.) rufa obscuriventris* var. *aggerans* Wheeler, Proc. Amer. Acad. Arts Sci., 52:540.
1940 *F. (F.) rufa clivia* Creighton, Amer. Mus. Nov., 1055:8.

Records: Spirit Lake. Also Okoboji (F. S. Stancliffe).

The erect hairs of this variant seem rather deciduous. Therefore single workers are not easily identified. The nests found by the writer were under rocks banked with plant debris. This variant is apparently rare in Iowa, as it is more properly a member of Merriam's Transition Zone. The writer has not seen any specimens from Iowa which he considers intergrades between *clivia* and *obscuriventris,* although, according to Creighton (1940a), they occur in Minnesota.

10. *Formica (Formica) fossaceps* Buren

1942 *F. fossaceps* Buren, Iowa State Coll. Jour. Sci., 16(3):402-405. ☿ ♀ ♂

Type locality: Winterset.

The temporary host of this species is probably *F. fusca subsericea.*

11. *Formica* (*Formica*) *prociliata* Kennedy and Dennis

1937 *F. prociliata* Kennedy and Dennis, Ann. Ent. Soc. Amer., 30:531. ☿ ♀ ♂

Records: Sabula, Bellevue, Winterset, Denison, Inwood.

This species lives in fairly populous colonies and constructs a low, flattened mound of earth about 2 or 3 feet in diameter. At Gotham, Wisconsin, the author found a female of *prociliata* which had been adopted by a depauperate colony of *F.* (*neoformica*) *pallidefulva nitidiventris*. *F. nitidiventris* may, therefore, be considered as the host or at least an alternate host of *prociliata*.

12. *Formica* (*Formica*) *dakotensis montigena* Wheeler

1904 *F. montigena* Wheeler, Bull. Amer. Mus. Nat. Hist., 20:374. ☿ ♀ ♂
1913 *F.* (*F.*) *dakotensis* var. *montigena* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:391, 394, 463. ☿ ♀ ♂

Record: Cherokee County (Prof. H. E. Jaques' Nat. Hist. Survey).

The writer has not taken specimens of this species from Iowa but has seen a specimen belonging to this form and collected in Iowa in the National Museum.

13. *Formica* (*Formica*) *reflexa* Buren

1942 *F. reflexa* Buren, Iowa State Coll. Jour. Sci., 16(3):399-402. ☿ ♀ ♂

Record: Spirit Lake.

Several colonies of this interesting species were taken at the above locality. In each case the colony consisted of only a few *reflexa* workers but numerous workers of the host, *F. fusca subsericea*. As the writer has stated (1942), this species may possibly be a nondulotic, permanent social parasite. This type of parasitism has been hitherto unknown in the genus *Formica*.

14. **Formica (Formica) knighti** n. sp.

WORKER. Length of major worker, 7.5 mm.

Head, excluding mandibles, about as broad as long, with posterior border feebly excised in the middle, the posterior corners rounded, and sides slightly convex; scarcely narrower in front than behind. Mandibles 8-toothed. Clypeus rather angularly produced. Frontal area small, much wider than high. Frontal carinae evenly diverging, their length equal to twice the diameter of the antennal foramina. Eyes hairy. First funicular joint one-fourth again as long as the second, the second slightly longer than the third, and each joint to the penultimate in turn slightly longer than the succeeding, the second almost one-half again as long as the penultimate. Promesonotal outline strongly convex. Mesoepinotal impression deep and wide in large workers; marked by sutures before and behind. Declivity of epinotum a little longer than the base, meeting the latter with an angle of approximately 120–130 degrees. Petiole cuneate in profile, anterior and posterior faces weakly convex. Superior border

rather sharp; seen from behind angularly produced upward but usually notched at the tip.

All surfaces opaque except the frontal area, which is smooth and shining, and the mandibles, which are moderately shining and longitudinally striate.

Erect hairs numerous, short, bristle-like, yellow, usually pointed at the tip but on the thoracic dorsum and gaster sometimes blunt or slightly clavate. Hairs present on all regions, even a few on the cheeks; few, however, on the gula. The numerous hairs on the scapes and legs short and strongly oblique or subappressed. Pubescence dense in all regions.

Ground color of head and thorax yellowish red, but usually heavily infuscated with black, even in the largest workers. Smaller workers have the head and thorax nearly as black as the gaster.

Described from numerous workers taken from a single nest near Bonaparte, July 13, 1941. The nest was located in pasture land covered with a rather dense growth of scrub oaks. The nest was well hidden under low bushes, and considerable plant debris had been used in the construction of a low dome, immediately under which were numerous workers and the brood.

This species has about the same coloration as *F. postoculata* Kennedy and Dennis but does not seem closely related to it. *F. postoculata* has no hairs on the eyes, and no pilosity on the scapes or tibiae. It is much smaller in size, and there are several other differences in pilosity and in the shape of the head and thorax.

F. knighti appears most closely related to *F. impexa* Wheeler, which it strongly resembles in the number and arrangement of the hairs. *F. knighti* may be distinguished immediately from *impexa* by the color of the head and thorax, which is deep red in *impexa* and scarcely infuscated except in the smaller workers. The head of *impexa* is less robust, more slender, and narrower in front; the clypeus is less produced and is rounded in front; the thorax appears less robust, and the mesoepinotal constriction is shallow and narrow; the petiole is blunter and more rounded when seen from behind; the erect hairs are blunt or clavate, and the hairs on the scapes and legs are blunter and erect. The erect hairs on the gaster are more numerous and larger and more conspicuous in *impexa*. The pubescent hairs also seem a little denser but shorter on *impexa*. The eyes of *impexa* are not distinctly hairy as in the new species. *L. knighti*, incidentally, is one of the few microgynous species with hairy eyes.

Since the queen is unknown, there is no actual evidence that *knighti* is a microgynous species, but its general habitus and close resemblance to *impexa* lead the writer to believe so. It is certainly distinct from any species in the *rufa* group known to the author. *F. knighti* would perhaps key down to *F. oreas* Wheeler in Wheeler's key to the *Formica* (1913), but workers of *oreas* may be distinguished immediately by the extremely abundant, very fine white hairs covering all parts. Many other differences show that *oreas* is not closely related to *knighti*.

F. knighti is probably a temporary social parasite of *F. fusca subsericea*.

I take great pleasure in dedicating this species to Dr. H. H. Knight, Professor of Entomology, Iowa State College.

In the author's unpublished thesis, this species had a manuscript name.

15. **Formica (Formica) microgyna** subsp. **spatulata** n. subsp.

WORKER. Length of largest worker, 7.0 mm.

Head a little longer than broad, with slightly convex posterior border and sides, somewhat narrower in front than behind. Clypeus subangularly produced. Basal funicular joints longer than penultimate joints. Pro- and mesonotum moderately convex. Mesoepinotal impression shallow. Epinotum rounded, the declivity rather gently sloped. Petiole narrow, blunt, and angularly produced upward, the apex sometimes truncated or notched, however.

Nearly all surfaces opaque. Frontal area shining. Erect hairs short, spatulate, becoming very wide and flattened toward the apex; rather abundant on nearly all surfaces, including the occipital corners. Not present on scapes or tibiae. The tips of the hairs appear somewhat frayed under high power of the binoculars. Pubescence dense and fine on all parts, adding to the opaque appearance.

Head and thorax orange-red to brownish red, apparently depending on the age of the individual. Gaster black.

FEMALE. Length, 5.7 mm.

Head much smaller than in major worker, posterior border more rounded. Eyes a little smaller in absolute size, but larger and more convex in proportion to the head. Thorax narrower than the head, elongate. Epinotum rather sloping. Petiole much as in the worker.

Less opaque than in the worker. Erect hairs spatulate but much longer than in the worker; present on the same regions. Pubescence dense.

Ground color of head and thorax yellowish red, but these regions, especially the dorsal surfaces, rather infuscated. Gaster black. Wings pale.

MALE. Length, 7.0 mm.

Mandibles pointed, edentate. Thorax narrower than the head. Petiole blunt, not or only slightly notched. Erect hairs somewhat more abundant than in the female, a little shorter, and only feebly spatulate. Pubescence sparser than in the female. Color black, with tibiae and tarsi yellowish. Wings pale hyaline.

Two nests of this form were found under rocks along the shore of a small lake near Spirit Lake, Iowa (type locality). Also included in the type series are some specimens (workers and females) from Wheaton, Minnesota.

This ant seems to be another geographical race of the widely distributed *Formica microgyna* Wheeler. *Spatulata* is more slender-bodied than most of these variants, the promesonotal outline is less convex, and the epinotum is more obtuse. The hairs are quite short and are more widened and flattened toward the apex than in any of the *microgyna* variants seen by the author.

From typical *microgyna, spatulata* may be easily distinguished by the lack of erect hairs on the scapes and the more spatulate hairs on the body, as well as the more slender body, less convex promesonotum, and more obtuse epinotum. From *microgyna rasilis,* with which it is perhaps most closely related, *spatulata* may be distinguished by the more numerous erect hairs and their presence on the occipital corners of the head, as well as the body proportions mentioned above. The same differences will also apply to *F. querquetulana* Kennedy and Dennis.

The female of *spatulata* is somewhat more slender-bodied than females of *microgyna, microgyna rasilis,* and *querquetulana,* and the same differences in pilosity mentioned for the workers are applicable. The petiole of the *spatulata* female is narrower than that of the *querquetulana* female.

F. microgyna spatulata may be distinguished immediately from all other Iowa species of *Formica* by the beautiful, dull, orange-red color of the head and thorax, which is especially striking in the younger workers.

The temporary host is *Formica fusca subsericea* Say, specimens of which were found in one of the Spirit Lake nests.

16. *Formica* (*Formica*) *indianensis* Cole

1940 *F. indianensis* Cole, Amer. Midl. Nat. 23:224-226. ☿ ♂

Record: Oak Grove State Park.

The author has taken 24 workers from at least two nests on a virgin prairie remnant at Oak Grove State Park. Stray workers were picked up, but the actual nests were not found and must have been small and well hidden.

The writer has taken a series of workers from a single nest at Inwood, Iowa, which shows all possible intergradations with *F. nepticula. F. indianensis* must therefore be closely related to *nepticula* in spite of their dissimilar appearance.

F. indianensis is probably a temporary social parasite of *F. fusca subsericea* or possibly *F. fusca argentea.*

17. *Formica* (*Formica*) *nepticula* Wheeler

1905 *F. nepticula* Wheeler, Bull. Amer. Mus. Nat. Hist., 21:270. ☿ ♀ ♂
1913 *F. (F.) nepticula* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:394, 396, 475. ☿ ♀ ♂

Record: Denison.

The nests of this species are usually located in and under small rotting limbs; some plant debris is used. Winged females were found in a nest in July.

18. *Formica* (*Formica*) *difficilis* Emery

1893 *F. rufa difficilis* Emery, Zool. Jahrb. Syst., 7:651. ☿ ♀ ♂
1913 *F. (F.) difficilis* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:395, 477. ☿ ♀ ♂

Records: Boone, Ames, Bellevue.

On a smaller scale, the nest architecture is like that of *F. rufa obscuriventris*. *F. diffcilis* is more timid and less aggressive than many ants of the *rufa group*. Its host is undoubtedly some form of *F. pallidefulva* Latreille.

19. *Formica (Formica) exsectoides* Forel

1886 *F. exsectoides* Forel, Ann. Soc. Ent. Belg., 30(C.R.):38. ☿ ♀
1913 *F. (F.) exsectoides exsectoides* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:396, 481. ☿ ♀ ♂

Records: Inwood, Denison, Mt. Vernon.

This species does not seem to thrive well in Iowa. The mounds that the writer has seen were rather small and scarcely conical. *F. exsectoides* often lives in huge aggregate colonies consisting of numerous mounds. In Iowa the writer has been unable to find more than a single mound in any one locality.

20. *Formica (Formica) ulkei* Emery

1893 *F. ulkei* Emery, Zool. Jahrb. Syst., 7:653. ☿
1913 *F. (F.) ulkei* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:396, 485. ☿ ♀ ♂

Record: Spirit Lake.

F. ulkei is apparently not common in any part of its range, and in Iowa must be very rare even in the northern part. The colony found by the writer was rather depauperate.

21. *Formica (Formica) sanguinea aserva* Forel

1901 *F. sanguinea aserva* Forel, Ann. Soc. Ent. Belg., 45: 395. ☿ ♀
1913 *F. (Formica) sanguinea aserva* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:389, 404. ☿ ♀ ♂
1925 *F. (Raptiformica) sanguinea aserva* Emery, Gen. Insec., fasc. 183:230.

Record: Rice Lake State Park.

This form has a rather boreal distribution. The writer has found it to be common in Minnesota and Wisconsin, but it seems rare even in the northern portions of Iowa. This ant is very fierce and aggressive but does not have dulotic habits. Its favorite nesting sites are old rotting stumps, a certain amount of plant debris being used around the base and in the large cavities.

22. *Formica (Formica) sanguinea rubicunda* Emery

1893 *F. sanguinea rubicunda* Emery, Zool. Jahrb. Syst., 7:647. ☿ ♀
1913 *F. (Formica) sanguinea rubicunda* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:390, 406. ☿ ♀ ♂
1925 *F. (Raptiformica) sanguinea rubicunda* Emery, Gen. Insec., fasc. 183:230.

Records: Dennison, Ames, Sabula, Oak Grove State Park, Clinton.

This ant is much more common in woodlands than *subintegra* but does not live in cities or towns. This is another example of how civilization has changed the fauna, reducing the numbers of some species, increasing those of others.

23. *Formica (Formica) sanguinea subintegra* Emery

1893 *F. sanguinea rubicunda* var. *subintegra* Emery, Zool. Jahrb. Syst., 7:648. ☿ ♀
1913 *F. (Formica) sanguinea subintegra* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:390, 410. ☿ ♀ ♂
1925 *F. (Raptiformica) sanguinea subintegra* Emery, Gen. Insec., fasc. 183:260.

Records: Ames, DeWitt.

This form seems to thrive well in lawns in cities and towns, unlike *rubicunda*, which is never found in such a situation. It is common within Ames, and the writer has also seen it at Clinton.

24. *Formica (Formica) sanguinea subnuda* Emery

1895 *F. sanguinea rubicunda* var. *subnuda* Emery, Zool. Jahrb. Syst., 8:335. ☿
1913 *F. (Formica) sanguinea subnuda* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:389, 409. ☿ ♀ ♂
1925 *F. (Raptiformica) sanguinea subnuda* Emery, Gen. Insec., fasc. 183:260.

Record: Sioux City (C. N. Ainslie).

Represented by six specimens found in the collection of the late C. N. Ainslie.

The epinotum is angulate in these specimens as in the preceding forms. They do not agree in this particular with Emery's description of *subnuda* (1894), and therefore may not actually be *subnuda*. For the present the writer prefers to regard them as such. The pilosity is the same as that of *aserva*, but *aserva* has a much darker colored and broader head.

25. *Formica (Proformica) neogagates* Emery

1893 *F. fusca subpolita* var. *neogagates* Emery, Zool. Jahrb. Syst., 7:661. ☿ ♀ ♂
1913 *F. (P.) neogagates neogagates* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 536. ☿ ♀ ♂

Records: Ames, Tama, Spirit Lake.

A rather rare species. Only two small nests have been found under stones near Ames. The Tama and Spirit Lake records are from single specimens whose nests could not be located.

The specimens listed as *neogagates* in the writer's preliminary list (1941a) are *neogagates vinculans*.

26. *Formica (Proformica) neogagates vetula* Wheeler

1895 *F. lasioides* var. *picea* Emery, Zool. Jahrb. Syst., 8:335. ☿ (nom. praeocc.)
1912 *F. (P.) neogagates lasioides* var. *vetula* Wheeler, Psyche, 19:90. (nom. nov.)
1913 *F. (P.) neogagates lasioides* var. *vetula* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 540. ☿

Records: Ames, Rice Lake State Park, Strawberry Point, Decorah, Inwood.

This ant seems to be the commonest form of *neogagates* in Iowa. It lives in small colonies in woodlands.

27. *Formica* (*Proformica*) *neogagates vinculans* Wheeler

1913 *F. (P.) neogagates* var. *vinculans* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 539. ☿ ♀

Records: Ames, Inwood.

This ant is rather common in lawns in Ames, a situation where the typical *neogagates* does not occur. The nests are rather small but more populous than the nests of typical *neogagates* seen. The ants will swarm out to defend their nests if provoked.

28. *Formica* (*Proformica*) *neogagates morbida* Wheeler

1913 *F. (P.) neogagates* var. *morbida* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:400, 538. ☿ ♀

Type locality: Lennox (P. J. Schmitt).

The writer does not possess specimens of this form but has seen the types in the Museum of Comparative Zoology at Cambridge, Mass.

29. *Formica* (*Neoformica*) *pallidefulva incerta* Emery

1893 *F. pallidefulva schaufussi* var. *incerta* Emery, Zool. Jahrb. Syst., 7:655. ☿ ♀ ♂
1913 *F. (N.) pallidefulva schaufussi* var. *incerta* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 554. ☿ ♀ ♂

Records: Ames, Clinton, Tama, Holy Cross, Rice Lake State Park, Inwood.

A common form in Iowa. There is considerable variation in color in the Iowa specimens. Some are as light as *pallidefulva schaufussi* Mayr, others as dark as *pallidefulva nitidiventris*. Nevertheless, the Iowa specimens almost always have the erect hairs lacking on the gula, and so all have been referred to *incerta*. The writer is convinced that *nitidiventris* can always be distinguished from incerta by its lack of both gular and petiolar hairs, and by its shorter, blunter gastric hairs, in spite of the frequent convergence in color.

30. *Formica* (*Neoformica*) *pallidefulva dolosa* Wheeler

1904 *F. pallidefulva schaufussi* var. *meridionalis* Wheeler, Bull. Amer. Mus. Nat. Hist., 20:370. ☿ (nom. praeocc.)
1912 *F. pallidefulva schaufussi* var. *dolosa* Wheeler, Psyche, 19:90. (nom. nov.)
1913 *F. (N.) pallidefulva schaufussi* var. *dolosa* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 554. ☿ ♀

Record: Glenwood.

This is a southern variant which apparently has managed to creep its way northward into Iowa only along the Missouri River bluffs.

F. pallidefulva dolosa is the only *Formica* which was found living on these bluffs. This is what one would expect if the Missouri River bluffs really have a southern fauna as has been contended. The genus *Formica* is poorly represented in the South.

31. *Formica (Neoformica) pallidefulva nitidiventris* Emery

1893 *F. pallidefulva nitidiventris* Emery, Zool. Jahrb. Syst., 7:656. ☿ ♀ ♂
1913 *F. (N.) pallidefulva nitidiventris* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 555. ☿ ♀ ♂

Records: Ames, Sabula, Oak Grove State Park, Princeton.
A common woodland form.

32. *Formica (Neoformica) pallidefulva fuscata* Emery

1893 *F. pallidefulva* var. *fuscata* Emery, Zool. Jahrb. Syst., 7:656. ☿ ♂
1913 *F. (N.) pallidefulva nitidiventris* var. *fuscata* Wheeler, Bull. Mus. Comp. Zool. Cambridge, 53:401, 557. ☿ ♀

Records: Ames, Clinton, Sabula, Holy Cross.
This form may have no validity other than as a mere color variety of *nitidiventris*. The females can scarcely be separated.

7. Polyergus

KEY TO SPECIES OF POLYERGUS

1. Gaster pubescent 2
   Gaster smooth and shining; pubescence very sparse ..... 1. *P. lucidus*
2. Gaster red like the head and thorax ..... 2. *P. rufescens breviceps*
   Gaster black ..... 3. *P. rufescens bicolor*

1. *Polyergus lucidus* Mayr

1870 *P. lucidus* Mayr, Verh. Zool.-bot. Ges. Wien, 20:952. ☿ ♀ ♂

Record: Backbone State Park.
This species probably has its western limit in Iowa. The slave of the colony found at Backbone State Park was *F. (Neoformica) pallidefulva incerta*. The ants listed as *lucidus* in the writer's preliminary list are *rufescens breviceps*.

2. *Polyergus rufescens breviceps* Emery

1893 *P. rufescens breviceps* Emery, Zool. Jahrb. Syst., 7:666. ☿

Records: Ames. Also Sioux City (C. N. Ainslie).
This ant is fairly common in lawns in Ames, and the writer has seen it also within Clinton, Des Moines, and Davenport. It does not seem to occur, or at least must be very rare, outside city limits. In this peculiar preference it parallels *Formica sanguinea subintegra*.

3. *Polyergus rufescens bicolor* Wasmann

1901 *P. rufescens bicolor* Wasmann, Allg. Zeitschr. f. Ent. Neudamm, 6(N):23. ☿ ♀ ♂

Record: Backbone State Park.
A single female with a swollen gaster was taken at Backbone State

Park. She had been adopted by a medium-sized nest of *Formica fusca subaenescens*. Colonies found by the writer at Akeley and Jenkins, Minnesota, also had the same species as the slave.

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THE SEASONAL HISTORY AND HOSTS OF THE AMERICAN DOG TICK, DERMACENTOR VARIABILIS, IN IOWA[1]

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Biological studies on the American dog tick were started at the Tama Indian Reservation, Tama, Iowa, in April, 1941, and terminated in December; supplementary collecting was done at Ames during January, February, and March, 1942. This paper presents a summary of the data obtained.

Biological data concerning this tick were reported by Morgan (1899), Hunter and Hooker (1907), Banks (1908), Hooker (1908, 1909), and Hooker, Bishopp, and Wood (1912). These last authors recorded the adult ticks as occurring on 19 species of animals. They collected no larvae known to be of this species but found nymphs on the fox squirrel and swamp rabbit. Hadwen (1913) published data on the life history, and Hewitt (1915) gave an account of Canadian ticks but added little to Hadwen's information concerning *D. variabilis*.

The white-footed woodmouse, *Peromyscus leucopus*, was shown by Larrousse, King, and Wolbach (1928) to serve as a host for immature forms. Further information on taxonomy, distribution, seasonal history, and hosts was furnished by Cooley (1932, 1938). Bishopp and Smith (1938) published the most complete report, especially on the host relationship of the immature forms; and additional information on seasonal history and hosts was supplied by MacCreary (1940-1941).

This tick was recognized as a species for about 90 years before it was incriminated as a vector of human diseases. With it, Maver (1911) experimentally transmitted the western strain of Rocky Mountain spotted fever, and Dyer, Badger, and Rumreich (1931) showed it to be a vector of the eastern strain. Naturally infected specimens of the tick were collected in Virginia by Badger (1932). The tularemia organism was recovered from naturally infected ticks by Green (1931), and survival of this bacterium through stage-to-stage and generation-to-generation development of ticks was shown by Philip and Jollison (1934). The American dog tick is now considered to be the principal vector of Rocky Mountain spotted fever in the eastern United States. It was also incriminated in the transmission of bovine anaplasmosis by Rees (1932).

According to Jordan (1937), an outbreak of spotted fever occurred among the Indians at the Sac and Fox Reservation, Tama, Iowa, begin-

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ning in 1933; the prevalence of ticks in the area has been reported several times. Studies on *D. variabilis* were undertaken on the Reservation, and were restricted to this locality.

The Tama Indian Reservation is composed of about 3,300 acres. In 1936 the total cordwood was 4,000,000 feet b.m., made up of soft maple, cottonwood, red and white elm, mixed oaks, basswood, walnut, hickory, boxelder, hackberry, honey locust, and ash, listed in the order of their timber volume. Parts of the area were in plantations of black ash (18.4 acres), Norway and white pine (45.2), and soft maple, cedar and walnut (4.9). Important cover for rodents and other wildlife was formed by clumps of trees and shrubs found mostly in sparsely covered areas. In general, the Reservation varies from a hilly, sparsely forested, and well-drained condition in the north, to a flat, densely covered, and semi-swampy condition where the Iowa river traverses it in the southern portion.

The dog serves as the principal host for the adult stage of *D. variabilis*. According to Bishopp and Smith (1938), the female requires from 5 to 13 days for engorgement. Although no experiments were undertaken concerning this, general observations on engorgement periods support these findings. Bi-weekly examinations of dogs were made in order to assure the removal of ticks before repletion and to obtain seasonal history data. Occasionally a replete specimen was found, but it was considered to have been missed at the previous examination. Some of the dogs were checked for a short period and then disappeared, but a few were available for examination throughout the entire course of the investigation. Dogs that were "deticked" periodically throughout the spring, summer, and fall presented very interesting individual records.

No special effort was made to examine larger domestic animals during the active tick season. There were a number of horses on the Reservation, but few cattle. These domestic animals were examined closely during September, October, and November, or when ticks became scarce.

Field mice are considered the most important hosts for the immature forms and for this reason were live-trapped for seasonal tick activity and host records. A single-catch metal type of trap with a length of 5½ inches and a width of 4 inches was used. A small wad of cotton was placed in each trap as nesting material and for aid in detection of engorged ticks dropped from the mice. Metal traps are known to cause a high mortality of mice from heat during the summer and by low temperatures in the winter. However, little mortality will occur during the summer months if the traps are kept in the shade; during the colder months it is difficult to control this factor when metal traps are used. In order to catch both diurnal and nocturnal animals, the traps were kept set both day and night. The northern white-footed mouse is nocturnal, whereas the literature concerning the activity of species of the genus *Microtus* is conflicting. The latter are said to forage chiefly during the day, but their activity probably depends to some extent on the amount of cover present. That the type of trap used was suitable for *Peromyscus* was evidenced by the re-

capture of a large percentage of the mice, some of them a number of times, depending upon the length of time the traps remained in the same area. Whether the trapping method was satisfactory for the other species present is not known; some species are no doubt more difficult to trap than others. Mice were deticked in the field, ear-tagged, and released at the point of capture. The traps were set from 2 to 4 rods apart, either in a grid pattern or in a straight line, according to the vegetation present. When the ticks or mice became scarce in one area the traps were usually moved a distance greater than the known range of the mice.

Other animals such as cottontail rabbits, woodchucks, ground squirrels, and birds were shot, bagged, and taken to the laboratory for examination.

DISTRIBUTION

The American dog tick is reported to be common in certain parts of southern Canada. It is known to occur throughout the United States east of the Rocky Mountains, and is prevalent in parts of California. In the southern and eastern half of Iowa, this tick is well represented, according to the Iowa State Department of Health reports. Although apparently more numerous in these areas, it is no doubt present in other suitable habitats of the state. Hosts for the adult and immature stages are known to occur throughout Iowa.

ADULT ACTIVITY

Adult specimens have been collected on animals in Texas at all times of the year, according to Hooker, Bishopp, and Wood (1912). Hadwen (1913) states: "As soon as the snow disappears and the warm weather begins, adults are apparently found everywhere." Cooley (1932) mentions the inactivity of the adults during the winter months in the North. Bishopp and Smith (1938) report that in Maryland and adjacent states the ticks begin to appear from the middle of March to the middle of April, depending upon the temperature. They also state that few ticks are seen after August 1. Specimens have been collected in Oklahoma by the senior author as early as March and as late as September. Though no extensive search was made during the colder months, the adults are probably inactive during the winter period in that state. The adults are found on dogs in Delaware from April 29 through August 23, according to MacCreary (1941).

Several attempts by means of the "drag" or "flag" method were made at Ames, Iowa, to collect adults. No specimens were taken by this method. One male was taken on April 8, and a male and female on April 12, from debris on the ground. No dogs had been examined in areas where ticks were known to occur before the above dates. Six males and two females were removed from two dogs examined on April 16, at the Indian Reservation at Tama. One of the females was about three-fourths replete. These records seem to indicate that the adults of *D. variabilis* were active during the first week in April, but perhaps to a limited extent. Bi-weekly

examinations of dogs were made thereafter in an attempt to obtain seasonal history data only. No effort was made to collect large numbers of ticks, but certain dogs were deticked regularly and thoroughly. As mentioned above, some of the dogs were checked throughout the course of the work. It was thought that a decrease in the population of ticks might occur if specimens were removed from the dogs over such a period of time. However, this would probably be true only if the animals remained at home. Most of the dogs "strayed" from home either during the day or at night. Dogs that ran at large were more heavily infested than those remaining at home. To avoid undue local reductions in tick populations, certain dogs were left untagged and were never deticked. Those animals were included in examinations made in August, September, and November. They also served the purpose of increasing the number of examinations when ticks became scarce.

A summary by months of the number of ticks collected from dogs is given in Table 1. The average number per dog and the percentage of dogs infested are given. Since the work started on April 16, only 13 animals were examined during that month. The average number of ticks per dog increased from 40.53 in April to a peak of 89.17 in May, with a

TABLE 1

A Monthly Average of Ticks (*D. variabilis*) Collected From Dogs, April 16, Through December 21, 1941, and the Percentage Infested

Month	No. Dogs Examined	Total No. of Ticks	Adult Ticks		Average No. of Ticks	Percentage of Dogs Infested
			Males	Females		
April	13	527	327	200	40.53	100
May	68	6,064	3,139	2,922	89.17	100
June	111	3,138	1,631	1,483	28.27	100
July	151	1,582	759	807	10.47	94.0
August	118	104	37	61	.88	41.5
September	251	26	11	12	.10	6.4
October	248	1	1	0	.004	.4
November	116	0	0	0	0	0
December	56	0	0	0	0	0
Totals	1,132	11,442	5,905	5,485		
Mean average					(10.10)	(49.14)

marked decrease for the months thereafter. The last replete female was taken on September 18. This specimen was noted at the previous examination and was left for engorgement. Only one male specimen was removed (Oct. 9) from the 248 animals checked in October. No ticks were found on animals examined in November or December. For the season, a total of 5,905 males and 5,485 females were taken. The females were more active during the rapid population decline of July, August, and September. A very interesting "natural" decrease of ticks was noted when the average number per dog fell from 89.17 in May to 28.27 in June, but the percentage infested remained the same. A few nymphs and

larvae were collected from dogs; these will be discussed below in the section which treats of those stages. Adult ticks were removed from the following animals: cow, horse, pig, raccoon, fox squirrel, house cat, and woodchuck.

Concerning hosts of the immature stages, very little has been published. Hunter and Hooker (1907) failed in an attempt to feed larvae on cattle. Hunter and Bishopp (1910) state that the young stages are found upon various small mammals, but mention no particular species. Nymphs were reported collected from the fox squirrel and swamp rabbit by Hooker, Bishopp, and Wood (1912). They induced larvae to attach to a bovine, but did not succeed in feeding that stage on dogs. The white-footed woodmouse, *Peromyscus leucopus*, was reported as a host for the larvae and nymphs by Larrouse, King, and Wolbach (1928). The most important data concerning hosts of immature stages were published by Bishopp and Smith (1938). Their work is quoted because of its importance: "The following is a list of the collections of immature stages contained in the accession catalogue. The number of times nymphs and larvae have been taken on the respective hosts is as follows: whitefooted mice (*Peromyscus*): larvae 68, nymphs 18; meadow mice (*Microtus*): 12, 13; pine mice (*Pitymys*): 4, 8; house mouse (*Mus domesticus*): 3, 0; kangaroo mouse (*Zapus*): 1, 1; mouse, species in doubt: 5, 5; cottontail rabbit: 3, 8; swamp rabbit: 2, 1; cotton rat (*Sigmodon hispidus*): 3, 1; Norway rat: 2, 1; wood rat (*Neotoma*): 0, 1; squirrels: 0, 7; cat: 0, 2; shrew (*Blarina brevicauda*): 2, 1; sheep: 0, 1 (unengorged); cattle: 0, 1 engorged; mole (*Scalopus acquaticus machrinus*): 1 unengorged, 0. The larger number of collections of ticks from *Peromyscus* as compared with those from *Microtus* was due to the much larger number of *Peromyscus* collected." MacCreary (1940) states that the meadow mouse is the preferred host of the spotted fever tick in Delaware. Similar observations were made by the same author the following year, and he found a number of other mice infested as well. Larval and nymphal stages were taken from the domestic rat and cottontail rabbit, but no specimens were found on the few birds he examined.

The average numbers of larval and nymphal ticks collected from the northern white-footed mouse are presented below in Table 2. This species was greatly predominant among mice on the Reservation. More than 95 per cent (2,656) of the mice trapped were of this species. The increase in the number caught, from 286 in July to 496 in August, was probably due in part to an increase in the number of traps employed. Since the work was started on April 16, the early spring emergence of the young ticks was not ascertained in 1941. Several of the mice trapped in April were infested with more than 100 larvae, one with 186. Some ticks were probably lost from the latter mouse, since it was examined several hours after death. The average of 32 larvae per mouse in April far exceeded that of any other month. The number gradually decreased from 3.36 in May to .01 in November. The last larva was taken on November 23. No specimens were taken from the 238 mice examined in December, January, and February. The mice trapped in January, February, and March

TABLE 2

A Monthly Average of Larvae and Nymphs (*D. variabilis*) Collected From the Northern White-footed Mouse, *Peromyscus leucopus noveboracensis*, From April 16, 1941, Through March 31, 1942, and the Percentage of Mice Infested

Month	No. Mice Examined	Total No. of Ticks	Average No. per Mouse		Percentage of Mice Infested
			Larvae	Nymphs	
April, 1941	40	1,290	32.05	.20	90.00
May, 1941	149	609	3.36	.72	78.53
June, 1941	168	425	1.25	1.27	69.04
July, 1941	268	594	1.09	.98	60.48
August, 1941	496	1,170	1.99	.36	56.04
September, 1941	490	425	.73	.11	39.59
October, 1941	450	124	.24	.02	18.00
November, 1941	299	6	.01	.007	1.67
December, 1941	191	0	0	0	0
January, 1941	17	0	0	0	0
February, 1942	30	0	0	0	0
March, 1942	40	15	.35	.02	.12
Totals	2,638	4,658			

were taken at Ames, Iowa. The first larvae collected in the spring of 1942 were taken on March 24. Five of the six mice examined thereafter were infested. Even though replete nymphs were collected on April 17, the average was far below that of the larvae during April and May, 1941. They were slightly more numerous in June, but were less active during the latter part of the summer and fall. The last specimen was collected on November 8. As with the larvae, no specimens were taken in December, January, and February. The first nymph collected in the spring of 1942 was taken on March 31. The immature stages collected in March were taken from areas where trapping had been done during January and February. It is likely that inactive larvae and nymphs were present in those areas during January and February.

It can be seen from the above table and by records from the cottontail rabbit (Table 3) that the larvae were more active in April than at any other time. Few nymphs were present during April, but they increased to a peak in June. This was evident not only on *Peromyscus,* but on practically all the other animals listed in Table 3. That the immature forms are inactive during the winter months is indicated by the number of mice examined with negative results during that period, and by the fact that mice examined in the same areas in March were infested. Bishopp and Smith (1938) collected larvae and nymphs on field mice in the District of Columbia and nearby Maryland and Virginia during the winter months. They also state that seed ticks were relatively scarce in the spring, but nymphs were present in large numbers.

The number of larval and nymphal ticks collected from some of the other animals is presented below in Table 3. Some of the data are insignificant but are given, since more information is needed concerning hosts of immature stages.

TABLE 3

NUMBERS OF LARVAL AND NYMPHAL TICKS (*D. variabilis*) COLLECTED FROM VARIOUS ANIMALS, APRIL 16, THROUGH DECEMBER 21, 1941

Month	Meadow Mouse, *Microtus p. pennsylvanicus*			Prairie Harvest Mouse, *Reithrodontomys megalotis dychei*			Fox Squirrel, *Sciurus niger rufiventor*			Rabbit, *Sylvilagus floridanus mearnsi*			House Cat, *Felis domestica*			Dog, *Canis familiaris*		
	No. Exam.	L*	N*	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N	No. Exam.	L	N
April	0	0	0	0	0	0	0	0	0	13	249	2	0	0	0	13	0	0
May	4	38	4	1	0	0	8	2	0	10	68	9	6	151	2	68	1	2
June	6	3	23	2	0	0	4	3	7	5	2	36	7	23	2	111	1	23
July	0	0	0	2	0	0	4	0	0	4	2	0	4	7	3	151	1	15
August	1	21	0	10	4	1	3	1	1	5	26	5	4	7	1	118	4	2
September	0	0	0	26	1	0	3	0	0	5	2	0	1	0	1	251	3	0
October	0	0	0	34	2	0	1	0	0	2	0	0	0	0	0	248	0	0
November	0	0	0	11	0	0	1	0	0	6	0	0	0	0	0	116	0	0
December	6	0	0	6	0	0	0	0	0	6	0	0	0	0	0	56	0	0
Totals	17	62	27	92	7	1	24	6	8	56	349	52	22	188	8	1132	10	42

* L = Larvae; N = Nymphs.

Four records obtained are not included in Table 3. A nymph was taken from the woodchuck, *Marmota m. monax,* in May, and three flat larvae were removed from the Virginia opossum *Didelphis v. virginiana,* on August 31. One larva was removed from the house mouse, *Mus. m. musculus,* in August, and one larva was taken in July from the prairie jumping mouse, *Zapus hudsonius campestris.* Eleven woodchucks, two opossums, one house mouse, and two prairie jumping mice were examined.

Meadow mice were either difficult to trap or were present only in small numbers. The 17 animals examined yielded 27 nymphs and 62 larvae. However, six of these mice were trapped in December when no ticks were active. The records tend to indicate the abundance and seasonal trend of the immature ticks. If the number trapped is an index of the number present, meadow mice probably are not of great importance as hosts on the Reservation.

The prairie harvest mouse is even less important than the above species. One nymph and seven larvae were collected from the 92 trapped mice. Because of small amount and short length of hair this mouse affords little protection to the ticks that do attach.

Eight nymphs and six larvae were removed from the 24 fox squirrels examined. Nymphs have been collected from fox squirrels by different workers, but Bishopp and Smith (1938) record no larvae.

The larvae were more active in April as evidenced by the number of specimens collected from the cottontail rabbit. The seasonal trend of both larval and nymphal stages on the cottontail is similar to that on the northern white-footed mouse and in part, that on other animals. The 56 animals examined yielded 52 nymphs and 349 larvae. One rabbit shot on the laboratory grounds was infested with engorging immature ticks. It appears that the cottontail rabbit, at least under certain conditions, might be a more important host than is generally thought.

There were a number of house cats on the Reservation, but these were difficult to examine. It is unfortunate that at least a few were not checked in April, since 151 larvae were found on six cats during May. One of these was infested with 97 larvae. A total of 8 nymphs and 188 larvae were removed from the 22 cats examined. Bishopp and Smith (1938) record no larvae from the house cat.

The writers found in the literature no previous larval or nymphal records from the dog. A total of 10 larvae and 42 nymphs, representing 43 infestations, were collected, however, from 1,132 dogs examined at Tama, Iowa. The ticks were in all stages of development, many of the nymphs fully engorged. The engorged specimens had probably been overlooked at the previous examination. Flat specimens are difficult to find, especially larvae, and no doubt many were overlooked. A thorough examination in search of young ticks could not be given each dog, because of the amount of time involved.

Parker (1937) states that in *D. variabilis* territory there is a higher proportion of spotted fever cases in women and children than in men because of the close association of this tick with the home through the

agency of dogs. It would be interesting to know just how many cases of this disease have been the result of immature ticks completing their development on rabbits, house cats, dogs, or other animals that range near the home. There is little doubt that the American dog tick can complete its entire life-cycle in the "backyard."

PLACES OF ATTACHMENT

It is known that some ticks are not only specific for certain animals, but are specific as to the parts they parasitize. The areas of attachment may afford complete or incomplete protection for the tick.

No definite data were obtained on places of attachment for the adult stage of the dog tick; no doubt the greatest numbers attach about the head, neck, and shoulders, and along the back.

Some figures were obtained on the attachment of the larval and nymphal forms. Those data perhaps are not of great scientific value, but are of interest. They represent observations made from July through December. The percentages of larvae and nymphs infesting the various parts of the body are as follows: Larvae: ears, 71.07; cheeks, 10.08; other parts of the head, 8.49; back and shoulders, 5.74; neck, 3.60; legs, .73; sides and belly, .27; Nymphs: back and shoulders, 36.80; neck, 22.67; head (except ears and cheeks), 19.70; cheeks, 13.38; ears, 6.69; sides, .74. In the case of larvae, almost 90 per cent of the ticks were removed from the head, and 71 per cent of the total were found on the ears. Few specimens were found on the posterior part of the body. Infestations of the tail were noted several times in April when the ticks were numerous, but not after that date. In the case of nymphs, only about 40 per cent were found on the head and 6 per cent on the ears. Almost 60 per cent were found on the neck and along the forepart of the back. In general, it may be said that the larvae show a preference for the head, especially the ears, and that the nymphs tend to congregate about the neck and shoulders and forepart of the back.

SUMMARY

Seasonal history and host studies on the American dog tick, *Dermacentor variabilis*, were conducted at the Tama Indian Reservation, Tama, Iowa. The work started in April, 1941, and was terminated in December. Some collecting was done at Ames during January, February, and March to determine the spring emergence of the immature stages.

Bi-weekly examinations of dogs were made to determine the seasonal trend of adults, and mice were live-trapped for data on the seasonal history of young ticks. Other animals were checked at every opportunity.

Results of the "drag" and "flag" method at Ames and the collecting done at Tama, indicate the adult ticks began activity the first week in April. The average number of adult ticks per dog each month was approximately 40, 89, 28, 10, .8, .1, and .004, from April through October, respectively. The last specimen was taken on October 9. One hundred per cent of the dogs were infested during April, May, and June. A total

of 1,132 dogs were examined. No new host records for adults were obtained, but a number were collected from the horse, cow, pig, fox squirrel, woodchuck, house cat, and raccoon.

The work was started too late to determine the spring emergence of the larvae and nymphs in April, 1941. The average number of larvae removed from the northern white-footed mouse, *Peromyscus leucopus novoboracensis,* was approximately 32, 3, 1, 1, 1, .7, .2, and .01, and that of nymphs was .2, .7, 1.2, .9, .3, .1, .02, and .007, in the months of April through November, respectively. The last nymph was collected on November 8, and the last larva on November 23. In January and February, 1942, 47 mice were examined at Ames, but no ticks were found.

The 40 mice checked in March yielded 14 larvae and 1 nymph. The first larva was collected on March 24 and the first nymph on March 31. The ticks collected in March were taken from the same area where trapping had been done in January and February. These results seem to indicate that the immature stages are not active during the winter months in Iowa. A total of 2,656 white-footed mice, 92 prairie harvest mice, 19 meadow mice, 2 prairie jumping mice, and 1 house mouse were examined. The northern white-footed mouse appears to be the most important cricetine on the area. Results indicate that the prairie harvest mouse is not a very favorable host. Data on the other three species were too incomplete to warrant conclusions.

Larvae and nymphs were also collected from several other hosts. The total number of animals examined and the number of larvae and nymphs removed are as follows: dogs, 1,132 examined, 10 larvae and 42 nymphs; cottontail rabbits 56, 349 larvae, 52 nymphs; house cats 22, 189 larvae, 8 nymphs; fox squirrels 24, 6 larvae, 8 nymphs; woodchucks 11, 0 larvae, 1 nymph; opossums 2, 3 larvae, 0 nymphs. Many of these animals were examined when the ticks were either scarce or inactive.

Some information was obtained on the attachment of the larval and nymphal stages of the American dog tick on mice. The percentage infesting the various parts of the body are as follows: larvae: ears, 71.07; cheeks, 10.08; other parts of the head, 8.49; back and shoulders, 5.74; neck, 3.60; legs, .73; sides and belly, .27; nymphs: back and shoulders, 36.80; neck, 22.67; head (except ears and cheeks), 19.70; cheeks, 13.38; ears, 6.69; sides, .74. In general, larvae show a preference for the head, especially the ears, whereas nymphs tend to congregate about the neck, shoulders and forepart of the body.

More than 4,394 animals were examined during the course of the investigation. Included were 266 birds (many ground-inhabiting species) and a number of horses, cattle, and domestic chickens. Some small mammals not mentioned above were also examined, but with negative results.

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THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

III. Some Phosphorus Relationships of *Chaetomium funicola*[1]

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INTRODUCTION

The necessity of phosphorus to the development of fungi has been recognized and repeatedly demonstrated since the early investigations of Pasteur (81, 82, 83, 84) and Raulin (87). Although the role that phosphorus assumes in the activity of fungi is still obscure, except with certain yeasts, the possible close relationship of phosphorus to carbohydrate dissimilation for some fungi might be inferred from the increasing demonstrations of phosphorylation in the higher plants (34, 35, 41, 52, 107), yeasts (18, 50, 55, 65, 66, 70, 86), bacteria (5, 79, 116), and animal tissues (4, 16). With such a possible relationship in mind, experiments were conducted with *Chaetomium funicola* Cke. to determine (a) the extent and duration of phosphorus removal from Czapek-Dox medium by developing cultures of the fungus; (b) the formation of a non-orthophosphate P fraction in the medium; (c) the nature of the acid-soluble phosphorus fractions in the mycelium; (d) the interrelationship of the acid-soluble phosphorus fractions in the mycelium through use of macerated mycelial preparations; (e) the respiratory behavior of such mycelial preparations; and (f) the formation of phosphoglyceric acid, uptake of phosphorus, and the formation of methylglyoxal, pyruvic acid, and acetaldehyde by such preparations.

LITERATURE REVIEW

The literature on the phosphorus relations of fungi other than yeasts has been confined largely to *Aspergillus niger*, a fungus which is very different in behavior from *Chaetomium funicola*. A review of some of the available facts concerning this and other fungi is presented here to show the limited scope of the investigations of this subject. Although orthophosphate salts have been used almost exclusively in the cultivation of fungi and in a study of their phosphorus relationships, Coupin (17) and Dox (20) early determined the equal availability to *Aspergillus niger* of

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organic phosphate and inorganic ortho-, pyro-, and metaphosphate salts, the non-availability of hypophosphate, and the toxicity of phosphite and hypophosphite salts. Schnücke (92) obtained the best yields of *A. niger* mycelium on liquid nutrient media containing 0.075 per cent initial concentration of KH_2PO_4. Koch and Reed (45), on the other hand, obtained progressively lower yields as the initial concentrations of KH_2PO_4 were lowered from 0.5 to 0.05 per cent. Results similar to the latter were obtained by Steinberg (96) for *A. niger* and by Tamiya (102) for *A. oryzae*. Vorbrodt (112) obtained the same ultimate yield of *A. niger* mycelium with 0.5 per cent and lower concentrations of KH_2PO_4 in the medium, except that at concentrations of 0.01 per cent KH_2PO_4 or lower, the yield was much reduced. *Chaetomium funicola* formed maximum yields of mycelium at initial concentrations of 0.1 and 0.025 per cent KH_2PO_4 (94). A close positive relationship between yields of *Aspergillus niger* mycelium and sugar consumed at different initial concentrations of phosphorus was noted by Braun and Erey (9). Waterman (113) noted a close relationship between the concentrations of phosphorus below the optimum and sugar consumed. Semeniuk (94) found greater utilization of sugar per unit dry weight of *Chaetomium funicola* mycelium at the high initial concentrations of phosphorus in the medium.

In relation to products formed by *Aspergillus niger*, Butkewitsch and Timofeeva (11, 12) obtained greater yields of citric acid (with no noticeable influence on oxalic and gluconic acid production) from cultures developing under growth-limiting concentrations of KH_2PO_4 than from cultures developing under adequate concentrations of phosphate for the maximum development of the fungus. Lvoff and Limberg (64) obtained more intense consumption of sugar with greater production of citric acid and later production of oxalic acid by mycelial mats in the presence of added KH_2PO_4. Gluconic acid production was depressed slightly by the presence of added phosphate. With cultures of *A. oryzae* initiated from spores in media containing M/20, M/40, M/200, and M/1,000 KH_2PO_4, Tamiya (102) observed marked lower yields of kojic acid with lower concentrations of KH_2PO_4 but ethanol was found only with M/20 and M/40 KH_2PO_4. Five-day-old mycelial mats placed on media of M/40, M/1,000, and M/0 KH_2PO_4 produced aerobically kojic acid and no ethanol and anaerobically much ethanol but no kojic acid. The presence of inorganic phosphate in the medium either slightly retarded or had no effect on the formation of these products since an abundance of phosphorus was considered to be already contained within the mycelium. With *Rhizopus* sp., Takahashi and Sakaguchi (101) observed ethanol production by submerged mycelium to be greater under lower than average concentration of phosphorus in the medium. Fumaric acid production by surface mycelial mats was greater with higher concentrations of phosphorus in the medium although the production of succinic, malic, and lactic acids remained unaffected.

The disappearance of phosphorus from the medium under developing cultures of *Aspergillus niger* has been accounted for by the total phosphorus appearing in the mycelium (69, 92). The percentage of phosphorus in the mycelium was found to be greater in the earlier stages of fungus

development than in the later stages (92, 112). Higher percentages of total phosphorus in the mycelium occurred with higher initial concentrations of KH_2PO_4 in the medium. Growth-limiting initial concentrations of KH_2PO_4 in the medium yielded mycelium with a stable, low value of 0.36 per cent total phosphorus as P_2O_5 (112). Maximum accumulation of phosphorus in the mycelium was reached at a period of abundant sporulation of the fungus (67, 69, 92) and subsequently decreased with liberation of phosphorus to the medium (67, 69). A reduction of 30 per cent of the phosphorus in *A. niger* occurred in one week of autolysis (69), and a 71 per cent decrease occurred under prolonged cultivation of the fungus for approximately one year (92). *Oidium lactis* and *Dematium pullulans* showed only a slight decrease.

The nature of the phosphorus accumulated in the mycelia of fungi was early noted by Iwanoff (39), for the Agaricaceae, to be organic phosphorus with only small amounts of inorganic phosphorus being present. Zeller (117) recognized these same two phosphorus groups for the higher fungi. He considered the organic phosphorus to be lecithin phosphorus.

Goupil (30) determined mineral, lecithin, and nuclein phosphorus in *Amylomyces rouxii*. Of the total phosphorus in *Aspergillus niger*, Koch and Reed (45) early determined 68.0 per cent extractive (water-soluble) phosphorus, 29.0 per cent protein (nuclein) phosphorus, and 3.0 per cent lecithin phosphorus. Similar values of 60–80 per cent for acid (aqueous) soluble phosphorus were obtained by Vorbrodt (112) and 60–70 per cent by Michel-Durand (69). Vorbrodt considered lecithin phosphorus to be present only in insignificant amounts, but Michel-Durand noted amounts of 5–17 per cent. The extractive or acid-soluble phosphorus as referred to by these latter two investigators was separated further into inorganic and organic phosphorus fractions. The organic phosphorus constituted 0–25 per cent of this fraction as observed by Vorbrodt and 39–67 per cent as determined by Michel-Durand. With yeasts, Macfarlane (65) observed that 50 per cent of the phosphorus contained in these cells was acid-soluble and consisted of 30 per cent inorganic orthophosphate P, 50 per cent labile phosphorus (pyrophosphate), and 20 per cent organic phosphorus. Lohmann (60, 61) found approximately 50 per cent inorganic orthophosphate P, 15–20 per cent pyrophosphate P and the remaining organic phosphorus.

The conditions surrounding the development of *A. niger* have been found to influence the amounts and relative proportions of the different phosphorus constituents in the mycelium. The quantities of acid-soluble and protein phosphorus in the mycelium reached a maximum at a time corresponding approximately to the maximum development (weight) of the mycelium (67, 69, 112). The maximum quantity was lower, however, as the initial concentration of phosphorus in the medium was lower (112). In terms of percentage of dry weight of mycelium, extractive phosphorus and protein phosphorus constituted fairly constant percentages under conditions of 0.5–0.05 per cent initial concentration of KH_2PO_4 in the medium, as observed by Koch and Reed (45). Lower initial concentrations of 0.01–0.005 per cent KH_2PO_4 in the medium resulted in lower per-

centages. Vorbrodt's data, on the other hand, showed progressively lower percentages of these same phosphorus fractions as the initial concentration of KH_2PO_4 was lowered from 0.5 to 0.01 per cent. The protein and acid-soluble phosphorus fractions in this latter work together constituted the total phosphorus in the mycelium, which means, as already pointed out, that the percentage of phosphorus in the mycelium decreased under these conditions.

The ratio of acid-soluble phosphorus to protein phosphorus was lower in the early period of fungus development (112), but such a relationship is not evident in Michel-Durand's data. Also, the influence of initial concentration of phosphorus in the medium on this ratio is not clear since Vorbrodt's data show slightly greater values at very low initial concentrations of 0.025 and 0.01 per cent KH_2PO_4 in the medium while Koch and Reed's data show the reverse.

Lipid phosphorus was found in only trace amounts by Koch and Reed (45) and Vorbrodt (112), but Michel-Durand found lipid phosphorus in the greatest amount at the time of complete sporulation of the mycelium when it represented 17 per cent of the total phosphorus present. A constant low value of 7 per cent was reached after a short period of autolysis.

The constituents of the acid-soluble phosphorus fraction in *A. niger* have been found to undergo considerable fluctuations in relative amount, although the fraction as a whole was found constantly present as 60–80 per cent of the total phosphorus. Since Vorbrodt and Michel-Durand considered only two components, inorganic and organic phosphorus, an increase in one meant a corresponding decrease in the other. Thus, considering only the organic phosphorus fraction, higher proportions of this fraction were obtained in the early stages in the development of *A. niger* up to the time of complete sporulation (69), followed by a decline on autolysis to a lower level. With lower initial concentrations of phosphorus in the medium, lower proportions of organic phosphorus were obtained in the mycelium which in one instance decreased to zero.

MATERIALS AND METHODS

The same culture of *Chaetomium funicola* was used as in the previous studies (93, 94). Other fungi used were: *Fusarium lini,* obtained as culture 1W from Dr. J. J. Christensen, University of Minnesota; *F. oxysporum* var. *cubense,* isolated by Mr. Clifford Meredith from infected banana trees in the West Indies; *F. bulbigenum* var. *niveum,* isolated from wilt-damaged watermelons at Muscatine, Iowa; and *Aspergillus niger,* isolated from apples stored at high temperatures at Ames, Iowa.

The same cultures already reported in experiments 2 and 3 of part 2 of the present series (94) served in the studies of phosphorus removal by developing cultures of *Chaetomium funicola.* Details for the preparations of these cultures were given therein.

The mycelium used in the remaining experiments was initiated from spores at 25–30°C. on Czapek-Dox medium in petri dishes (9 cm. diameter) with approximately 20 cc. medium per dish, maintained in the dark

usually for 6 days.[3] The mycelial mats were removed from the desired number of cultures, bulked on a cheesecloth, washed usually three to five times by immersion in as many changes of sterilized distilled water and compressed each time by hand to remove as much of the adhering liquid as possible. Further treatment of the mycelium as to period of storage at 33°F. varied with the different experiments as noted. Immediately before use the mycelium was macerated by grinding in a porcelain mortar with pestle with the aid of silica sand and water to obtain a soupy suspension which could be either (1) poured into a graduated cylinder, (2) easily drawn up into a wide bore pipette, or (3) drawn up into a hypodermic needle. No attempt was made to standardize the relative amounts of mycelium and water in making this macerated mycelial preparation except to add enough water to obtain a fairly thick but workable suspension. Therefore, the results between experiments were not directly comparable although within experiments they were. Steam sterilized utensils were used in all cases to reduce the sources of contamination to a minimum.

The analysis for acid-soluble phosphorus fractions in the mycelium was made on freshly harvested mycelium which was allowed to drain for approximately 10 minutes after bulking, squeezed by hand of adhering liquid medium, then taken to a cold room maintained at 33°F. and there washed three times in as many volumes of cold (33°F.) distilled water. The hand-squeezed mycelium was then placed in a porcelain mortar, flooded with an equal weight of 10 per cent trichloracetic acid solution, quartz sand added and ground by hand with a pestle to obtain a "soupy" suspension. This suspension was then made to volume with cold 5 per cent trichloracetic acid solution and allowed to remain in the cold for 4 hours. The liquid portion was then separated from the macerated mycelium by centrifuging and by several passages through filter paper. Phosphorus analyses were made immediately on this filtrate according to the methods indicated below.

Experiments on the interrelationship of the various acid-soluble phosphorus fractions within mycelial preparations were conducted at 30°C. in the dark. Measured volumes of macerated mycelial preparations were transferred to each of a number of previously arranged erlenmeyer flasks containing known volumes of test solutions. Volumes of 15 cc. of the suspension from each experimental flask were removed immediately and at spaced intervals of time thereafter and introduced into 5 cc. of 20 per cent trichloracetic acid solution. These samples were then placed in a 33°F. room where they were allowed to remain about two days[4] after all the samples had been taken from any one experiment (usually 24–48 hours' duration). The samples were then treated as a unit, being centri-

[3] Period for formation of a surface mycelial mat with sporulation beginning only with *Aspergillus niger*.

[4] Preliminary trials revealed constant inorganic orthophosphate P values with periods of extraction at 33°F. varying from several minutes to 5 days, while organic phosphorus values showed an increase reaching a maximum after 1 to 2 days' extraction. This latter behavior was obtained only with mycelial preparations which previously had not undergone an autolytic period in a water-toluene mixture (43).

fuged and filtered through filter-paper at the same time, and phosphorus determinations made on the filtrates.

Inorganic orthophosphate P was determined by King's colorimetric method (44) with a Dubosq colorimeter and electric light reflected from a white sheet of smooth paper. Total phosphorus was determined by conversion to inorganic orthophosphate by careful slow ashing in concentrated sulfuric acid over a low flame. Small additions of 30 per cent H_2O_2 were made occasionally to complete the oxidations. Pyrophosphate formed in this operation was converted into the ortho form by hydrolysis of the ashed sample in N HCl for 7 minutes in a boiling water bath after neutralization. Lohmann's method (59, 60, 61, 62) of acid hydrolysis was followed for the phosphorus soluble in the trichloracetic acid extract, and the phosphorus fractions determined according to the formula used by Macfarlane (65): Labile phosphorus $= \Delta(7' - 0') - \Delta(30' - 7')$; organic phosphorus = total phosphorus — (orthophosphate P at zero minutes hydrolysis + labile phosphorus).

Phosphagen phosphorus determination in the mycelium was made on the cold trichloracetic acid extract immediately following centrifugation. Eggleton and Eggleton's colorimetric and precipitation methods (22) were followed and the necessary precautions taken for temperature control as pointed out by Fiske and Subbarow (25).

The respiratory activity of macerated mycelial preparations was determined in a Warburg-Barcroft respirometer[5] shaken at 110 oscillations per minute in a waterbath maintained at 30.4° ± 0.01°C. Atmospheric air only was used in the studies. Small pieces of folded filter-paper were placed in the central cup of concentrated KOH solution in each chamber to facilitate absorption of CO_2. Observations for the position of the manometer fluid were made once within the first hour and usually every 2 hours thereafter. Aeration of the experimental mixtures in each chamber was provided only at the time of making an observation and readjusting the position of the manometer fluid by a 3-minute opening of the stopcock connecting each chamber to its manometer. No attempt was made to determine the weight of the mycelium introduced into each chamber except that the amount introduced was the same in any one experiment. Each chamber received 0.5 cc. of macerated mycelial preparation to which solutions of various substances were added to make a total volume of 1.5 cc.

Experiments on phosphoglyceric acid formation[6] and its detection as the barium salt were conducted according to the method of Neuberg and Kobel (72, 73) as modified by Vercellone and Neuberg (111) and by Stone (99). The latter worker observed that holding the flasks containing the experimental mixtures for several days at 5°C. increased the yield of phosphoglyceric acid. The mycelial particles were removed by centrifu-

[5] Grateful acknowledgment is here made to Dr. C. H. Werkman of the Bacteriology Department for permission to use this apparatus in his laboratory.

[6] Appreciation is here expressed to Dr. R. W. Stone, then of the Bacteriology Department, for his assistance in this phase of the work.

gation at the termination of the experiment and the centrifugate analyzed for the presence of the acid.

Analysis for phosphorus uptake by macerated mycelial preparations was made by determining the concentration of inorganic orthophosphate P by the Kuttner and Lichtenstein method (53) in the liquid portion of the experimental mixtures. Samples of the experimental mixtures were removed at intervals, filtered to remove the mycelial particles, and inorganic orthophosphate P determined immediately in their filtrates.

Tests for methylglyoxal, pyruvic acid and acetaldehyde formation by macerated mycelial preparations were carried out following the methods of Simon and Neuberg (95). Mycelial preparations were allowed to remain in suspension of prepared solutions for 48 hours at 28.5°C. at which time the mycelial fragments were centrifuged out and 20 per cent trichloracetic acid solution added to the centrifugate to make a final concentration of 5 per cent of the acid. Isolations of methylglyoxal, pyruvic acid and acetaldehyde were made as derivatives of 2: 4-dinitrophenylhydrazine hydrochloride added in a 2N HCl solution (0.5 gm. of the compound in 60 cc. of hot 2N HCl solution) in proportions of 10 cc. of the compound solution to 50 cc. of the centrifugate. Usually 2–3 hours were allowed for the formation of a precipitate. The precipitate was separated from the mother liquid and fractionated with solvents according to the method of Simon and Neuberg. In one trial methylglyoxal was determined quantitatively by the colorimetric method of Barrenscheen and Dreguss (3), using a standard methylglyoxal solution prepared according to Hoffmann and Neuberg (38) and standardized according to Kühn and Heckscher (51) and Fischler and Boettner (24).

EXPERIMENTAL RESULTS

1. *Removal of phosphorus from Czapek-Dox medium by developing cultures of Chaetomium funicola.*

The data for inorganic and total phosphorus in the medium and for total phosphorus in the mycelium under conditions of different initial concentrations of KH_2PO_4 and pH are presented in tables 1 and 2, respectively. With normal (0.1 per cent) or greater concentrations of KH_2PO_4, *C. funicola* continued to remove phosphorus from the medium over the entire period of 23 days. Varying the initial pH of the medium did not influence this behavior except under initially alkaline and very acid conditions, where the amounts of mycelium formed and phosphorus removed were retarded. Greater removal of phosphorus from the medium occurred with higher concentrations of phosphorus, yielding correspondingly higher total and percentage phosphorus contents in the mycelium. With 0.025 per cent initial concentration of KH_2PO_4 the inorganic orthophosphorus supply in the medium was depleted after approximately 13 days, but the plants still continued to produce mycelium. After 23 days as much mycelium was produced as in cultures on 0.1 per cent initial concentration of KH_2PO_4 which still retained much inorganic phosphorus in the medium. With no phosphate added to the medium, development of *C.*

TABLE 1

ANALYSIS OF *Chaetomium funicola* CULTURES DEVELOPED ON CZAPEK-DOX MEDIUM WITH DIFFERENT INITIAL CONCENTRATIONS OF KH_2PO_4

Days of Fungus Development	Initial Percentage Concentration of KH_2PO_4 in the Medium						
	0	0.025	0.1	0.3	0.6	1.5	3.0
Mycelium Formed, Milligrams							
6	0.6	60.0	82.3	79.4	87.6	64.5	56.2
13	35.7	287.6	267.8	260.8	233.2	185.4	209.2
22	40.8	400.8	418.0	339.3	355.3	354.4	333.4
Inorganic Orthophosphate P in the Medium, Milligrams							
0	0.0	1.8	7.0	21.1	42.6	104.5	211.3
6	0.0	1.4	5.9	19.8	40.9	106.6	210.6
13	0.0	Trace	4.1	15.3	36.7	99.7	196.1
22	Trace	0.2	3.9	13.8	33.6	97.8	187.2
Total Phosphorus in the Medium, Milligrams							
0	Trace	.....	8.0	21.9	43.0	106.6	210.0
6	1.0	1.8	6.5	19.6	41.2	107.0	208.4
13	0.7	0.8	5.3	18.0	38.7	103.9	205.5
22	0.7	1.0	5.7	16.4	33.7	91.0	181.4
Total Phosphorus in the Mycelium, Percentage							
13	0.3	0.7	0.8	1.0	1.6	1.8	3.9
22	.....	0.8	1.4	1.8	1.8	2.1	3.0

funicola was limited, suggesting the utilization of the phosphorus contained in the spores originally sown in the medium. Rennerfelt (88) found a higher percentage of phosphorus in the spores of *Aspergillus niger* than in the mycelium. Phosphorus constituted 5.77 per cent of the ash in spores and only 1.84 per cent of the ash in the mycelium.

Although the values for total phosphorus in the mycelium are not in complete agreement with the decrease in total phosphorus in the medium, the discrepancies obtained are similar to those already recorded for *Aspergillus niger* (69). In general, however, the phosphorus analyses in the mycelium support the observations already made of the disappearance of phosphorus from the medium.

2. *Formation of a non-orthophosphate P fraction in Czapek-Dox medium.*

The presence of a non-orthophosphate P fraction in the medium, as determined by difference between the values for total and inorganic orthophosphate, is suggested more convincingly by the data in table 2 than by those in table 1. Because of the necessity of varied dilutions of the media according to the concentration of phosphorus present, certain undetermined variable errors were introduced into the phosphorus values shown in table 1. In this experiment, in addition, no step was taken to reduce to orthophosphate any pyrophosphate formed during ashing of the sample in the determinations of total phosphorus. Accordingly, some of the total phosphorus values are lower than the corresponding inorganic phosphorus values. An explanation other than errors in dilutions cannot

TABLE 2

ANALYSIS OF *Chaetomium funicola* CULTURES DEVELOPED ON CZAPEK-DOX MEDIUM WITH DIFFERENT INITIAL pH VALUES AND WITH 0.1 PER CENT INITIAL CONCENTRATION OF KH_2PO_4

Days of Fungus Development	Initial pH of Culture Medium							
	2.90	4.03	4.98	6.05	7.20	7.78	8.15	8.68
Mycelium Formed, Milligrams								
10	27.5	211.8	195.8	183.1	173.8	99.8	45.9	16.3
23	33.0	410.7	270.6	355.9	521.5	208.6	383.6	180.2
Inorganic Orthophosphate P in the Medium, Milligrams								
0	7.5	7.3	8.1	8.1	7.8	7.5	8.1	7.0
10	6.8	5.2	4.7	5.7	6.0	6.2	6.5	6.8
23	5.5	2.3	2.3	2.1	2.9	4.2	3.6	4.2
Total Phosphorus in the Medium, Milligrams								
0	8.1	7.8	8.1	8.1	7.8	7.5	7.5	6.8
10	7.8	6.5	6.5	6.8	7.5	7.8	9.4	8.3
23	6.0	3.9	3.9	3.4	3.6	4.9	5.2	4.7
Total Phosphorus in the Mycelium, Percentage								
10		1.4	1.3	1.0	0.9	0.8	0.8	1.1
23	0.9	0.7	1.0	0.9	0.6	0.7	0.6	0.9

be offered for the reverse situation where inorganic orthophosphate P is lower than total phosphorus as obtained in the analysis at zero time. Non-orthophosphate P in the medium was indicated more consistently in cultures with low initial concentration of phosphorus in the medium. Furthermore, the liberation of non-orthophosphate P from the mycelium is suggested by the cultures with no additions of KH_2PO_4 and by the older cultures initially supplied with 0.025 per cent KH_2PO_4.

In the experiment represented by the data in table 2, a consistently significant non-orthophosphate P fraction was detected in the medium after 10 and 23 days of fungus development in contrast to the small positive and negative values attributable to errors in method obtained at zero days' development. Thus, while the net non-orthophosphate P fraction at zero time analysis represented 1 per cent of the total phosphorus present in the medium, at 10 and 23 days this fraction represented 20 and 21 per cent, respectively. The presence of a non-orthophosphate P fraction in the medium was further supported by the results of analysis by Lohmann's hydrolysis method of the phosphorus in the filtrate from 14-day-old cultures grown in petri dishes. This filtrate tested per cc. 115.0 gamma total phosphorus of which 95.6 gamma were inorganic orthophosphate P, 12.7 gamma labile phosphorus, and 6.7 gamma organic phosphorus. The organic phosphorus was hydrolyzed to the extent of 40 per cent in 3 hours. This observation was further confirmed by methods designed to precipitate and to characterize the organic phosphorus fraction.

In the first trial, adapting the method of Tanko (107), 300 cc. of Czapek-Dox solution filtrate obtained from 6-day-old petri dish cultures and made alkaline to phenolphthalein was treated in slight excess with

basic lead acetate. The precipitate was centrifuged off, washed several times with cold water, resuspended in water, and decomposed in an ice bath by additions of a dilute solution of H_2SO_4. The precipitate formed was centrifuged off and discarded. Barium hydroxide was then added to the centrifugate until no further precipitation occurred, and was followed by 2.5 volumes of 95 per cent ethanol. The precipitate was centrifuged off, washed successively with 70 and 95 per cent ethanol and dried at 50°C. Although most of this precipitate was made up of $BaSO_4$, a portion soluble in 0.1 N H_2SO_4 solution yielded a phosphorus fraction containing approximately 50 per cent inorganic orthophosphate P with the remainder being undetermined. Hexosediphosphate was not detected as a constituent of this undetermined phosphorus fraction.

In a second trial the same amount of solution used in the first was obtained from 18-day-old cultures. Of the total phosphorus present in solution, 24.7 per cent was in the non-orthophosphate form. The solution was made alkaline to litmus and treated in excess with basic lead acetate. The precipitate formed was washed three times by suspension in water and centrifuging, then suspended in water, decomposed with H_2S, and filtered. The filtrate was made alkaline to litmus, and the inorganic phosphorus precipitated with magnesium acetate and filtered out. The filtrate (80 cc.) was made acid by addition of 2 cc. glacial acetic acid, and 5 cc. of 20 per cent barium acetate solution were added. The precipitate was allowed to form over night at 33°F., centrifuged off, and washed in succession with 2 per cent glacial acetic acid, water, ethanol, and acetone. A light powder weighing 80.3 mgms. was obtained which gave a positive test for phosphorus and nitrogen, liberated H_2S on treatment with dilute H_2SO_4, and burned quickly to a metallic residue. Extraction of the powder with 0.1 N H_2SO_4 solution revealed 0.48 per cent total phosphorus in the powder, of which 44.0 per cent was inorganic orthophosphate P, 6.7 per cent labile phosphorus, and 49.3 per cent organic phosphorus. The organic phosphorus was hydrolyzed to the extent of 30 per cent in 30 minutes with no additional hydrolysis on further heating to 60 minutes. To the filtrate (87 cc.) yielding the above light powder were added 11 cc. of 95 per cent ethanol to make the concentration of the latter 10 per cent. No precipitate was formed. Ninety cc. of 95 per cent ethanol were then added and an abundant, flocculent precipitate was formed. The precipitation was allowed to proceed over night at 33°F. after which the precipitate was centrifuged off, washed in 50 and 95 per cent ethanol, and dried in air. A faintly yellow powder was obtained weighing 302.9 mgms. which gave a positive test for nitrogen and phosphorus and possessed an iodine-reducing power equivalent to 1.5 per cent glucose content. Barium made up 38.3 per cent of the weight of this powder while total phosphorus as extracted for the powder with 0.1 N H_2SO_4 made up 5.2 per cent. Organic phosphorus constituted 35.0 per cent and labile phosphorus 2.6 per cent of the total phosphorus, the remaining 62.3 per cent being inorganic orthophosphate P. Ninety per cent of the organic phosphorus in this fraction was resistant to 3 hours' hydrolysis by N HCl at 100°C.

3. *Nature of the acid-soluble phosphorus fractions in the mycelium.*

An analysis of the acid-soluble phosphorus in the mycelium of *C. funicola* and four other fungi is shown in table 3. The combined mycelia from 20 6-day-old petri-dish cultures for each fungus used, were adjusted to 100 cc. volume with a trichloracetic acid solution. The total acid-soluble phosphorus was definitely much higher with the three *Fusarium* spp. than with the other two fungi. The order of the fungi for total acid-soluble phosphorus in the mycelium from the least to the greatest was *Aspergillus niger, Chaetomium funicola, Fusarium lini, F. niveum,* and *F. cubense.* The amount of inorganic orthophosphate P varied with the different fungi according to the total acid-soluble phosphorus present, rep-

TABLE 3

ANALYSIS FOR PHOSPHORUS FRACTIONS IN THE TRICHLORACETIC ACID EXTRACTS OBTAINED FROM THE MYCELIA OF FIVE FUNGI

Time of Hydrolysis in N HCl at 100°C.	Orthophosphate P (Gamma) per 5 cc. of Acid Extract				
	Aspergillus niger	*Chaetomium funicola*	*Fusarium lini*	*Fusarium cubense*	*Fusarium niveum*
0	6.0	20.2	46.1	37.3	61.4
7	12.8	69.5	113.0	192.2	219.5
30	14.0	69.5	111.7	208.2	227.5
180	15.4	71.5	118.5	208.2	236.0
Total phosphorus	30.0	99.0	171.2	565.0	305.0
Inorganic orthophosphate P percentage	20.0	20.4	26.9	6.6	20.1
Labile phosphorus percentage	18.7	49.8	39.1	24.6	49.2
Organic phosphorus percentage	61.3	29.8	34.0	68.8	30.7
Percentage organic phosphorus hydrolyzed in 3 hrs.	12.7	6.8	9.5	8.2	26.2

resenting a fraction of approximately 20 per cent of the total. *Fusarium cubense,* however, contained inorganic orthophosphate P equal to only 6.6 per cent of the total acid-soluble phosphorus present. Labile phosphorus was 0.9, 2.4, 1.4, 3.7, and 2.4 times greater than the inorganic orthophosphate P for each of the fungi *Aspergillus niger, Chaetomium funicola, Fusarium lini, F. cubense,* and *F. niveum,* respectively. Together, the inorganic and labile phosphorus constituted 38.7, 70.2, 65.9, 31.2, and 69.4 per cent of the total phosphorus for each of the fungi, the remainder being organic phosphorus. The organic phosphorus for the most part was resistant to 3 hours' acid hydrolysis since for each of the above fungi the extent of hydrolysis attained was 12.7, 6.8, 9.5, 8.2, and 26.2 per cent. Phosphagen phosphorus was not detected.

The influence of age of *Chaetomium funicola* mycelium on the composition of the acid-soluble phosphorus fractions is shown by the data in table 4 and by figure 1. Washed and hand pressed mycelia from 40 petri dish cultures at two ages of development were made to 200 cc. with

TABLE 4

ANALYSIS FOR PHOSPHORUS FRACTIONS IN THE TRICHLORACETIC ACID EXTRACTS OBTAINED FROM MYCELIA OF 8- AND 18-DAY-OLD CULTURES OF *Chaetomium funicola*

Time of Hydrolysis in N HCl at 100°C.	Orthophosphate P (Gamma) per 2.5 cc. of Acid Extract	
	Age of Cultures	
	8 days	18 days
0	57.2	54.3
7	111.0	320.0
30	152.0	409.0
180	164.0	422.5
Total phosphorus	207.0	486.5
Inorganic orthophosphate P percentage	27.6	11.2
Labile phosphorus, percentage	6.2	36.3
Organic phosphorus, percentage	66.2	52.5
Percentage organic phosphorus hydrolyzed in 3 hours	68.6	75.0

trichloracetic acid solution. Total acid-soluble phosphorus was greater by 2.3 times in the 18-day-old mycelia than in the 8-day-old samples. Inorganic orthophosphate P was the same at both ages while labile phosphorus and organic phosphorus were greater by 5.9 and 0.8 times, respectively. As percentages of total phosphorus, inorganic orthophosphate P was lower by 60 per cent, labile phosphorus higher by 110 per cent and organic phosphorus lower by 27 per cent in the older mycelia. The percentage values of the total phosphorus for inorganic orthophosphate P, labile phosphorus, and organic phosphorus in the older mycelia were 27.6, 6.2, and 66.2, respectively. The hydrolysis curve suggests that the organic phosphorus fraction was similar in both ages of mycelium. This fraction liberated approximately 70 per cent of its phosphorus in 3 hours' hydrolysis, but 90 per cent of this was liberated during the first 30 minutes of hydrolysis. The possibilities are that, either the liberation of phosphorus from the labile phosphate fraction was incomplete after 7 minutes' hydrolysis, or the organic phosphorus fraction was composed of at least two fractions, one of which possessed a phosphorus labile to acid hydrolysis, the other a phosphorus that was quite stable.

4. *Interrelationship of the acid-soluble phosphorus fractions in the mycelium.*

The presence of the different acid-soluble phosphorus fractions in the mycelium of fungi suggested an investigation of a possible interrelationship like that recently found with yeasts, bacteria, blood cells, and various animal tissues. For this purpose use was made of macerated mycelial preparations of *C. funicola* and several other fungi prepared from freshly harvested 6-day-old mycelium to which different substances were added. The changes in phosphorus obtained at chosen intervals of time revealed

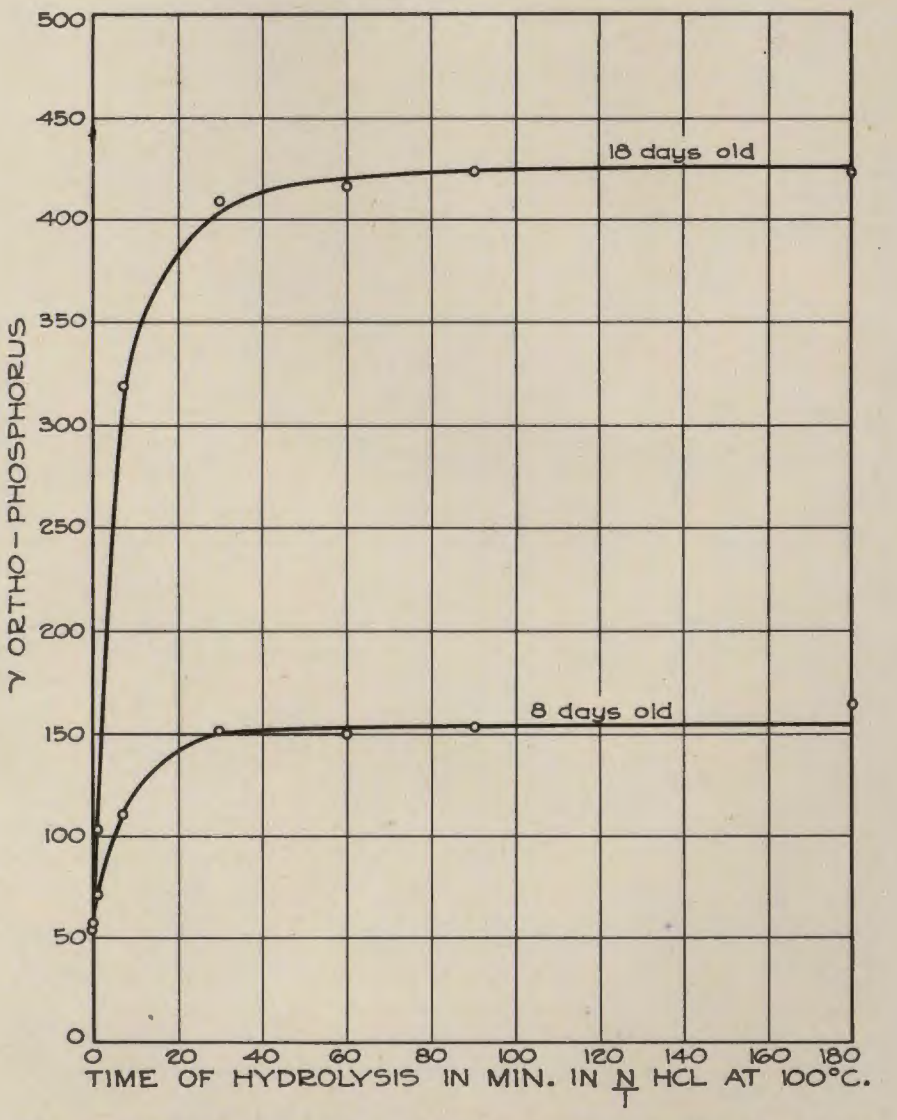

FIG. 1. Hydrolysis curves of the acid-soluble phosphorus fraction obtained from *Chaetomium funicola* mycelia of two different ages.

autolysis to be the predominating if not the exclusive reaction of these preparations under the conditions employed. Figure 2 presents the changes obtained in an initial experiment in inorganic orthophosphate phosphorus and pyrophosphate phosphorus. Inorganic orthophosphate phosphorus increased with time, reaching a maximum in approximately 20 hours in all the experimental treatments. Glucose retarded phosphorus mineralization in the absence of sodium fluoride and toluene but

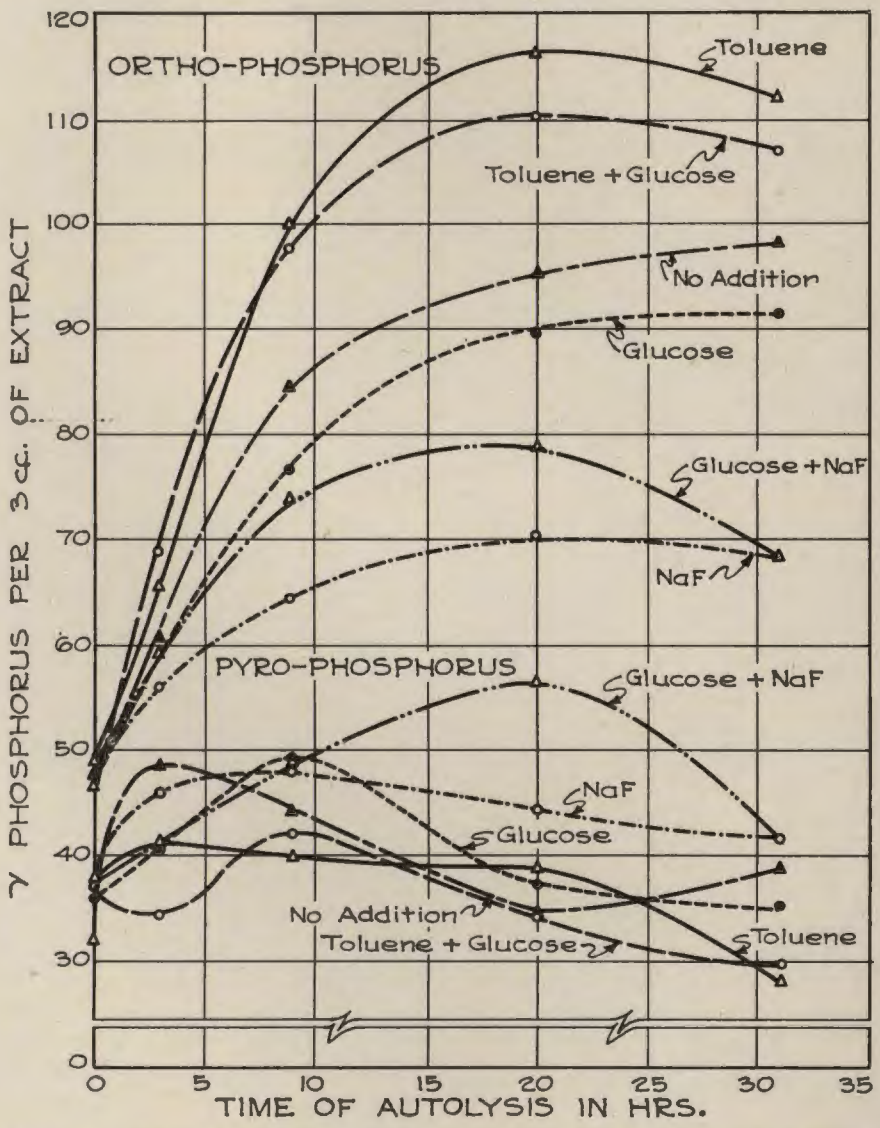

FIG. 2. Changes in two acid-soluble phosphorus fractions during autolysis of *Chaetomium funicola* mycelial preparations.

enhanced it in the presence of sodium fluoride and in the early period with toluene. Sodium fluoride exerted a retarding action, and toluene favored phosphorus mineralization in the absence and presence of added glucose. The pyrophosphate phosphorus fraction (7′–0′) increased with time and then decreased, except in the presence of toluene and glucose, where an initial decrease was obtained. The presence of glucose retarded by several hours the attainment of the maximum pyrophosphate values.

The maximal pyrophosphate values attained with no treatment or with toluene treatment were the same in the presence or absence of glucose, but were much greater in the presence of glucose with sodium fluoride.

TABLE 5

PROGRESSIVE CHANGES DURING AUTOLYSIS IN THE ACID-SOLUBLE PHOSPHORUS FRACTIONS OF MYCELIAL PREPARATIONS OF *Chaetomium funicola* * UNDER ADDITIONS OF TOLUENE AND NaF

Experimental Mixtures †	Phosphorus Fractions	Gamma Phosphorus per 2.5 cc. Acid Extract			
		Initial Values	Increases After Autolytic Periods (hrs.)		
			2¼	10	18
1. No additions	Total-P.....	113.0	10.0	17.5	22.1
	Inorg.-P.....	44.1	8.9	28.3	42.3
	Labile-P.....	30.9	− 2.9	− 4.0	− 4.9
	Organic-P-1‡.	16.2	− 2.8	−15.5	−16.2
	Organic-P-2§.	21.8	6.8	8.7	0.9
2. Toluene	Total-P.....	115.2	13.6	25.1	25.1
	Inorg.-P.....	41.2	7.4	50.5	62.9
	Labile-P.....	31.6	4.2	−16.3	−14.7
	Organic-P-1‡.	13.8	− 3.8	− 8.8	−12.3
	Organic-P-2§.	28.6	5.8	− 0.3	−10.8
3. NaF	Total-P.....	113.6	7.6	10.5	11.4
	Inorg.-P.....	45.4	10.6	20.1	31.4
	Labile-P.....	28.0	− 3.6	− 6.9	− 3.0
	Organic-P-1‡.	21.0	− 5.9	− 7.7	−15.2
	Organic-P-2§.	19.2	6.5	5.0	− 1.8

* Mycelium obtained from 78 petri dish cultures, grown 6 days, washed twice and macerated to a volume of 240 cc.

† Exp. mixture 1. 80 cc. mycelial preparation 160 cc. water
2. " " " 160 cc. water, 1 cc. toluene
3. " " " 160 cc. water, 48 cc. 0.2M NaF

‡ Organic phosphorus hydrolyzed by 3 hours' acid hydrolysis.

§ Organic phosphorus not hydrolyzed by 3 hours' acid hydrolysis.

Pyrophosphate accumulation was greater with sodium fluoride and lower with toluene than when both were present.

A comparison of the changes in the different acid-soluble phosphorus fractions in the presence and absence of toluene and sodium fluoride (Table 5) with and without glucose (Table 6) revealed progressive increases in total acid-soluble phosphorus and inorganic orthophosphate P, general decreases in labile phosphorus and 3-hour acid-hydrolyzable organic phosphorus, and initial increases with subsequent decreases in 3-hour acid-unhydrolyzable organic phosphorus. Maximal increases of 10–27 per cent and 70–200 per cent were obtained for total acid-soluble phosphorus and inorganic orthophosphate P, respectively. In general, toluene enhanced the rate and extent of phosphorus conversions while sodium fluoride exerted a retardation action. Glucose only slightly increased these conversions in the presence of these compounds.

In other experiments additions of inorganic orthophosphate buffer (pH 6.85), alone or in the presence of sodium fluoride or sodium fluoride plus glucose, did not alter appreciably the general behavior of the various

phosphorus fractions. Respiratory poisons, as potassium cyanide and ethyl urethane, phosphorylation-inhibiting phlorhizin, and iodoacetate, likewise exerted no noticeable influence on the changes obtained in the phosphorus fractions. Further, the same general behavior of phosphorus

TABLE 6

PROGRESSIVE CHANGES DURING AUTOLYSIS IN THE ACID-SOLUBLE PHOSPHORUS FRACTIONS OF MYCELIAL PREPARATIONS OF *Chaetomium funicola** UNDER ADDITIONS OF TOLUENE AND NaF WITH AND WITHOUT GLUCOSE

Experimental Mixtures†	Phosphorus Fractions	Gamma Phosphorus per 2.5 cc. Acid Extract					
		Initial Values	Increases After Autolytic Periods (Hrs.)				
			2½	5	8	12	17
1. NaF	Total-P.....	110.0	− 1.5	5.0	11.0	16.8	16.5
	Inorg.-P.....	34.0	6.4	10.8	16.6	27.5	34.7
	Labile-P.....	9.2	− 2.7	− 2.7	0.3	− 5.3	− 7.4
	Organic-P-1‡..	23.6	− 5.6	− 8.2	−13.7	−11.9	−14.5
	Organic-P-2§..	43.2	0.4	5.1	7.8	6.5	3.7
2. NaF plus glucose	Total-P.....	114.5	0.5	7.4	18.7	14.3	23.0
	Inorg.-P.....	34.1	8.1	14.3	21.9	38.0	50.1
	Labile-P.....	8.2	− 3.4	− 3.6	− 0.3	− 7.7	− 9.2
	Organic-P-1‡..	25.1	− 3.2	− 5.6	−11.1	−11.9	−16.1
	Organic-P-2§..	47.1	− 1.0	2.3	8.2	− 4.1	− 1.8
3. Toluene	Total-P.....	119.0	2.0	16.1	26.0	28.8	31.0
	Inorg.-P.....	38.5	18.4	29.8	44.7	63.0	76.7
	Labile-P.....	12.9	− 3.6	−11.2	−11.6	− 4.4	− 5.1
	Organic-P-1‡..	22.1	− 7.3	10.6	− 1.4	−15.0	−25.1
	Organic-P-2§..	45.5	− 0.5	−13.1	− 5.7	−14.8	−15.5
4. Toluene plus glucose	Total-P.....	117.2	15.1	18.8	27.8	29.8	32.3
	Inorg.-P.....	38.4	19.1	34.5	53.5	72.4	75.1
	Labile-P.....	7.7	− 0.7	− 2.9	− 5.8	1.0	1.3
	Organic-P-1‡..	23.6	− 8.5	− 1.8	− 9.2	−25.1	−22.7
	Organic-P-2§..	47.5	5.2	−11.1	−10.7	−18.5	−21.4

* Mycelium obtained from 80 petri dish cultures, grown 6 days, washed three times and macerated to a volume of 240 cc.

† Exp. mixture 1. 60 cc. mycelial preparation 12 cc. 0.2M NaF, 48 cc. water.
2. " " " 12 cc. 0.2M NaF, 18 cc. water, 30 cc. 10% [glucose.
3. " " " 1 cc. toluene, 59 cc. water.
4. " " " 1 cc. toluene, 29 cc. water, 30 cc. 10% glu- [cose

‡ Organic phosphorus hydrolyzed by 3 hours' acid hydrolysis.

§ Organic phosphorus not hydrolyzed by 3 hours' acid hydrolysis.

as with *C. funicola* was obtained with macerated mycelia of other fungi; namely, *Fusarium niveum, F. lini,* and *F. oxysporum* in the presence and absence of toluene or toluene plus sodium fluoride.

5. *Respiratory activity of macerated mycelial preparations of Chaetomium funicola.*

The foregoing mineralization of phosphorus in macerated mycelial preparations of *C. funicola* suggested a determination of the respiratory activity of such preparations as measured in a Warburg-Barcroft respiro-

TABLE 7

RESPIRATORY ACTIVITY OF MACERATED MYCELIAL PREPARATIONS OF *Chaetomium funicola**

Additions Made to Mycelial Preparations	Readings	Respiratory Quotient and cmm. CO_2 Evolved per Hour During Different Time Intervals (hrs.)												Total CO_2 Evolved
		0–1¼	1¼–4¼	4¼–7¼	7¼–9¼	9¼–10¾	10¾–12¼	12¼–14	14–15¼	15¼–16¾	16¾–18¼	18¼–20¼	20¼–22¼	22¼–24¼	
None	CO_2	20.4	13.8	25.5	66.9	104.0	44.5	24.0	26.5	29.0	31.8	30.0	33.4	26.3	1,019.9
	R. Q.	1.26	.95	.98	1.29	1.17	.77	.55	.58	.77	.87	.68	.92	.92	
Glucose	CO_2	20.0	16.5	34.1	86.4	111.0	135.0	110.0	119.3	113.0	144.7	97.5	80.0	27.9	1,859.1
	R. Q.	1.11	.94	1.02	1.13	1.37	1.25	1.23	1.25	1.24	1.85	1.23	1.01	4.88	
NaF M/300	CO_2	21.3	15.6	28.5	83.2	94.0	77.9	45.4	39.2	32.7	33.6	26.0	34.1	25.8	983.0
	R. Q.	1.13	.94	1.00	1.24	1.09	.80	.83	.87	.84	.90	.87	.93	.92	
Glucose+NaF M/300	CO_2	14.8	13.5	34.7	82.0	74.5	68.7	24.9	21.8	17.3	14.4	12.9	12.9	12.8	735.9
	R. Q.	1.32	.93	1.09	1.03	1.05	1.00	.74	.89	.88	.94	1.01	1.01	1.33	
Iodoacetate M/300	CO_2	23.5	14.8	23.4	37.3	42.3	52.7	50.1	59.6	52.6	50.6	46.8	46.8	30.7	927.5
	R. Q.	1.72	1.04	.94	1.07	1.11	1.11	1.08	1.11	1.03	1.03	.93	.97	.92	
Glucose+Iodoacetate M/300	CO_2	23.5	12.8	26.7	32.3	46.8	56.8	61.8	40.9	35.4	34.0	13.8	14.2	50.2	787.9
	R. Q.	2.18	1.11	1.02	2.07	1.09	1.10	1.01	.74	.68	.71	.52	.82	.74	
Oxalate M/300	CO_2	10.7	13.4	35.1	149.0	107.2	76.3	47.5	42.4	43.7	45.8	41.7	44.7	32.0	1,239.2
	R. Q.	1.25	.94	1.40	1.01	1.14	1.11	1.15	1.20	1.19	1.32	1.20	1.21	.86	
Glucose+Oxalate M/300	CO_2	14.5	15.8	52.1	107.8	132.8	162.2	128.5	124.5	118.5	108.0	107.5	107.5	79.1	2,188.9
	R. Q.	.97	.88	1.14	1.01	1.14	1.11	1.10	1.09	1.09	1.14	1.12	1.12	1.09	

* Fungus grown 6 days; mycelium freed of reducing sugars by washing in running tap water over a 12-hour period. Mycelial preparation made from 10 gm. moist material + 15 cc. water. Additions to mycelial preparation in each chamber: 0.5 cc. 15% glucose solution; 0.5 cc. 0.01M solutions of NaF, iodoacetate or oxalate, adjusted to pH 6.7, water to make 1.5 cc. total volume.

meter. Use was made of poisons of known action (7, 15, 19, 27) to learn something of the nature of the respiratory activity.

The respiratory activity data for mycelial preparations to which were

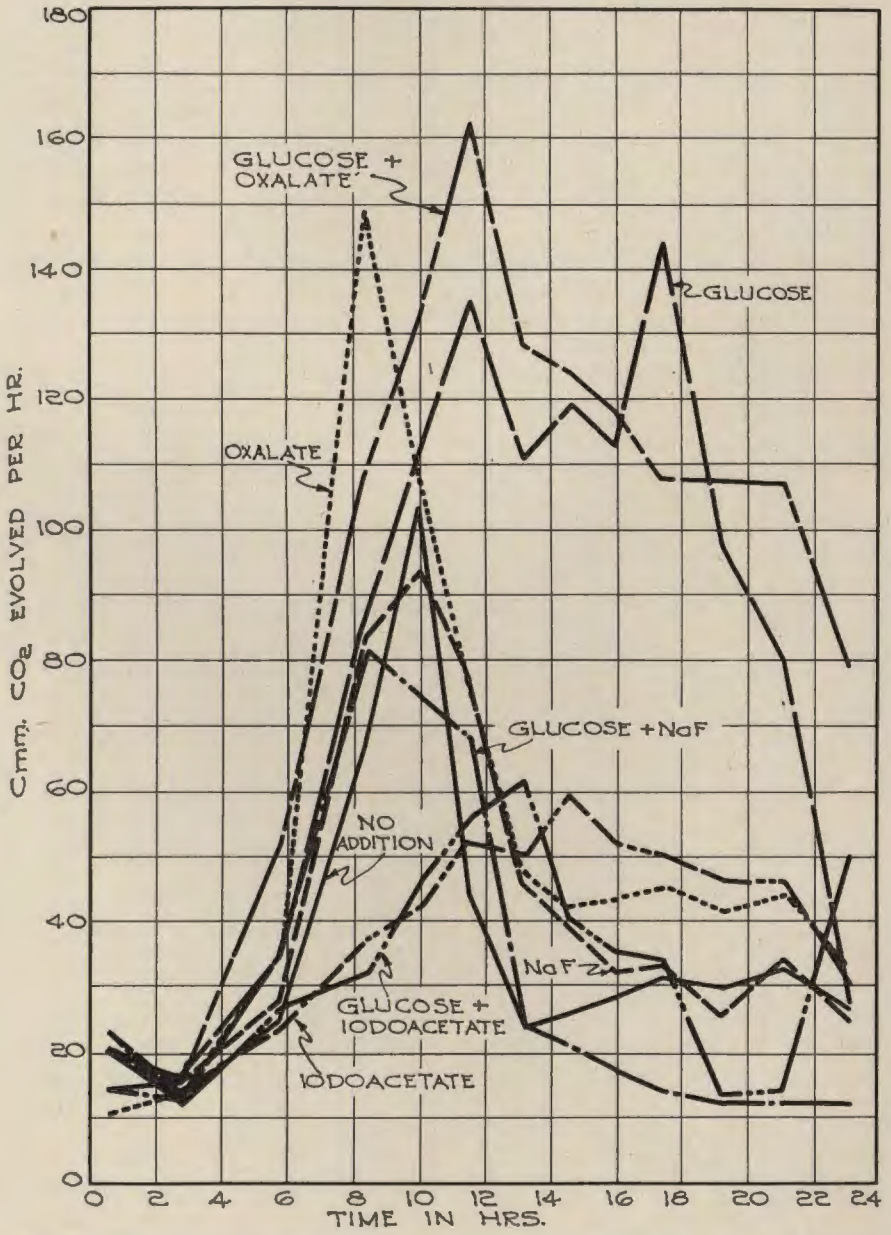

FIG. 3. Respiratory activity of macerated mycelial preparations of *Chaetomium funicola*.

added glucose, NaF, iodoacetate, and oxalate in several combinations are presented in table 7 and figure 3. These data reveal: (1) an initial induction period; (2) endogenous and exogenous respiratory activity (97, 98); (3) inhibition of exogenous activity by NaF and iodoacetate, with no effect on endogenous activity by NaF but with some inhibition by iodoacetate; and (4) slight stimulatory effect of oxalate on endogenous and exogenous activity.

The depressive effect of iodoacetate on endogenous activity and the complete suppression of exogenous activity was different from that reported for "*Fusarium* sp. H" (29) but in line with the concept that this compound interferes with the oxido-reduction processes (specifically, with triosephosphate dehydrogenase); more with fermentation than with respiration (110). Sodium fluoride is known to be a general inhibitor of esterase (including phosphatase) enzyme activity (21, 56, 57, 68) and particularly of the enolase enzyme converting 2-phosphoglyceric acid to 2-phosphopyruvic acid (114). The stimulatory effect of oxalate was in line with the suggestion (54) that it retards co-zymase destruction, although earlier work showed an inhibitory effect on glycolysis, specifically the conversion of phosphopyruvate to pyruvate and phosphate (63). Tamiya (102) reported a similar stimulatory effect for *Aspergillus oryzae*.

Further evidence for the distinction between endogenous and exogenous activities is supported by the effect of decreasing strengths of iodoacetate; namely M/30, M/100 and M/300 (Table 8). Complete inhi-

TABLE 8

IODOACETATE INHIBITION OF RESPIRATORY ACTIVITY BY MYCELIAL PREPARATIONS OF *Chaetomium funicola* *

Iodoacetate Concentration	Total cmm. CO_2 Evolved †	
	With Glucose	Without Glucose
0	559.9	128.7
M/30	4.6	0.0
M/100	28.7	10.3
M/300	60.2	103.6

* Fungus grown 13 days; mycelial preparation made from 10 gm. moist mycelium + 15 cc water. Additions to mycelial preparation in each chamber: 0.5 cc. 15% glucose solution 0.5 cc. iodoacetate solutions adjusted to pH 6.7 and water to make 1.5 cc. total volume

† Over a 6-hour period.

bition of exogenous activity was obtained with all strengths of iodoacetate, but inhibition of endogenous activity was milder with weaker strengths of the inhibitor.

Cyanide in strengths of M/1,000 and M/500 (Table 9) almost completely suppressed endogenous and exogenous activities (O_2 consumption to a slightly greater extent than CO_2 evolution) thereby suggesting that both processes are oxidative in nature. This effect on exogenous activity is different from that recorded for "*Fusarium* sp. H" (29) and yeast (98) where CO_2 evolution remained unaffected or affected to only a slight

TABLE 9

CYANIDE AND URETHANE INHIBITION OF RESPIRATORY ACTIVITY BY MYCELIAL PREPARATIONS OF *Chaetomium funicola* *

Poison Added	Concentration	Total cmm. CO_2 Evolved and O_2 Consumed †			
		With Glucose		Without Glucose	
		CO_2	O_2	CO_2	O_2
None	.....	568.2	555.4	130.0	117.0
KCN	0.001M	98.4	77.8	26.2	19.6
KCN	0.002M	58.8	41.3	14.0	14.0
Urethane	0.57M	31.9	29.6	45.8	.....

* Fungus grown 7 days; mycelium washed 3 times, let stand overnight at 33°F. then washed twice. Mycelial preparation made from 15 gm. moist mycelium + 20 cc. water. Additions to mycelial preparation in each chanber: 0.5 cc. 15% glucose solution, 0.5 cc. solutions of KCN and urethane and water to make 1.5 cc. total volume.

† Over a 12-hour period.

TABLE 10

INFLUENCE OF INORGANIC PHOSPHATE AND TOLUENE ON THE RESPIRATORY ACTIVITY BY MYCELIAL PREPARATIONS OF *Chaetomium funicola* *

Additions	Total cmm. CO_2 Evolved †	
	With Glucose	Without Glucose
None	1169.5	802.0
Phosphate	1066.3	1010.0
Toluene	18.2	52.5
Phosphate + toluene	35.9	30.9

* Fungus grown 6 days; mycelium washed free of reducing sugars and stored overnight at 33°F. Mycelial preparation made from 10 gm. moist mycelium + 15 cc. water. To mycelial preparation in each chamber added 0.5 cc. 15% glucose solution, 0.5 cc. phosphate buffer (2:1 of 2/3M K_2HPO_4 and 2/3M KH_2PO_4, respectively) pH 6.85 and water to make 1.5 cc. total volume. Six drops toluene added where indicated.

† Over a 14-hour period.

extent. A small residual exogenous activity over endogenous activity in the presence of cyanide might be indicative of a weakly anaerobic fermentative process such as has been found for a number of different fungi (109). Urethane 0.57M (Table 9) almost completely inhibited endogenous and exogenous activities in conformity with its behavior as a general poison of the dehydrogenase enzyme systems (7).

Additions of inorganic orthophosphate to macerated mycelial preparations (Table 10) resulted in an initial slight depression of endogenous and exogenous activities followed by sharp temporary stimulation. The duration of the depressive effect was longer with exogenous activity. This behavior with phosphate suggests a partially disorganized system undergoing autolysis analogous to that of yeast cells in contrast to an actively respiring maceration juice preparation (74). Further support in this direction may be derived from the nearly complete suppression of endo-

genous and exogenous activities by toluene either in the presence or absence of added inorganic phosphate (Table 10).

6. *Isolation of methylglyoxal, pyruvic acid, and acetaldehyde using macerated mycelial preparations of Chaetomium funicola.*

In a further endeavor to ascribe the functional significance of phosphorus in the mycelium of *C. funicola* to the phenomenon of phosphorylation in carbohydrate metabolism, repeated attempts were made to isolate phosphoglyceric acid, but these proved unsuccessful. Again in these instances use was made of macerated mycelial preparations obtained immediately before use from freshly harvested young (6-day-old) or old (14-day-old) mycelium or from harvested washed mycelium that had stood at 33°F. for several days. Alterations in certain conditions as time of incubation (up to 22 hours), temperature and the omission, concentration, and type of various substances pointed out by Stone (99) to be influential on phosphoglyceric acid formation in no way influenced the final result. Likewise, the course of phosphorus changes in the liquid portion of these experimental mixtures revealed no removal of inorganic phosphorus but rather a liberation similar to that already described. With these failures attention was then directed toward isolation of methylglyoxal, pyruvic acid, and acetaldehyde.

Preliminary experiments with macerated mycelial preparations from freshly harvested mycelium developed for 12 days in culture, or from a portion of it dried in vacuo over concentrated H_2SO_4 for several days, or another portion stored moist at 33°F. for 2 weeks yielded, in the presence of either hexosediphosphate or glucose with phosphate buffer (pH 6.87) and toluene, derivatives of 2 : 4-dinitrophenylhydrazine which on solvent fractionation (95) gave colors with alcoholic KOH characteristic for methylglyoxal, pyruvic acid and acetaldehyde and corresponding approximate melting points. In one trial methylglyoxal was found in nearly twice the quantity from hexosediphosphate as from glucose alone. Another trial was then made to obtain sufficient 2 : 4-dinitrophenylhydrazine derivatives to enable greater purification of the fractions. Five identical mixtures in each of five flasks were prepared with 3.3 gm. moist mycelium, 10 cc. 20 per cent glucose solution, 5 cc. phosphate buffer (pH 6.87), 25 cc. water, and 1 cc. toluene. After 48 hours' incubation, trichloracetic acid was added to make a 5 per cent concentration, filtered, and a solution of 2 : 4-dinitrophenylhydrazine added to the filtrate. Precipitation of 2 : 4-dinitrophenylhydrazine derivatives was allowed to proceed for 3 days at which time they were combined and fractionated. The results obtained were as follows:

Fraction	*Recrystallization medium*	*Melting point*	*Color with alcoholic KOH*
methylglyoxal	nitrobenzene	295°C.	blue violet
pyruvic acid	ethanol	211-214°C.	reddish brown
acetaldehyde	ethanol	167°C.	reddish brown

No mixed melting points were determined because the amount of ma-

terial obtained was small. Simon and Neuberg (95) record the same color reactions for these derivatives but with the following melting points: methylglyoxal, 298°C.; pyruvic acid, 216°C., and acetaldehyde, 164–165°C. Campbell (13) found 168°C. for acetaldehyde and 218°C. for pyruvic acid hydrazones.

DISCUSSION

As previously demonstrated (93) *Chaetomium funicola* converts the carbon of the glucose in Czapek-Dox medium mainly to carbon dioxide and mycelium. In consequence, it continues to form mycelium so long as there is sufficient undecomposed glucose remaining in the medium (94). The removal of phosphorus from the medium accordingly continues as mycelium is formed over a long period of time. In this respect *C. funicola* differs from *Aspergillus niger* but apparently is similar to *Oidium lactis* and *Dematium pullulans* (92).

The very high values for phosphorus in the mycelium of *Chaetomium funicola* grown on media with high initial concentrations of KH_2PO_4 correspond to similar high values reported for *Aspergillus niger*. The greater dissimilation of glucose per unit weight of mycelium found at the higher initial concentrations of KH_2PO_4 is considered due to the retarding influence of high phosphate concentrations on mycelium formation.

The origin of the non-orthophosphate fraction with its components of labile, easily and difficultly hydrolyzable organic phosphorus groups in the media of developing cultures of *C. funicola* may be identified with their liberation from ageing autolyzing hyphae. Schnücke (92) ascribed the apparent existence of an organic phosphorus fraction in the medium of *Aspergillus niger* cultures to errors in the methods of phosphorus analyses, since small negative and positive values were obtained. Braun and Frey (9) found organic phosphorus present in 27-day-old cultures of *A. niger* only on such media as showed an abundance of inorganic phosphorus remaining within the medium. More organic phosphorus (15–45 per cent of the total phosphorus present in the medium) was found in media initially supplied with organic forms of either or both nitrogen (as asparagin and peptone) and phosphorus (as phytin) than with inorganic forms of both. In the latter instances the organic phosphorus formed comprised only 4 per cent of the total phosphorus in the medium. Michel-Durand (69) and Mann (67) found organic phosphorus (2–28 per cent of the total phosphorus) in the medium only after the onset of autolysis, but the amount present was extremely variable and was explained in part by Michel-Durand by the presence of conidia of the fungus in the solutions being analyzed.

The findings of various acid-soluble phosphorus constituents in the mycelium of *Chaetomium funicola* and the other fungi here studied is in accordance with similar findings with yeasts (60, 61, 65), bacteria (79, 116), higher plants (34, 35, 115) and various animal tissues (76, 31, 60, 61, 62). While Vorbrodt and Michel-Durand considered only mineral and organic phosphorus fractions for *Aspergillus niger*, the present study has revealed the presence of inorganic orthophosphate P, labile phosphorus

hydrolyzable in 7 minutes at 100°C. in N HCl, and organic phosphorus of one or more components, one or more resistant to acid hydrolysis and another readily hydrolyzable by acid. Extremely labile phosphorus compounds of the type represented by phosphagen were not detected. The present results do not support Mann's observations (67) that the acid-soluble phosphorus in the mycelium is exclusively of the readily hydrolyzable type.

The significance of the phosphorus fractions in the mycelium of fungi has been variously construed. Koch and Reed (45) and Goupil (30) considered protein phosphorus and lecithin phosphorus to be the most important phosphorus constituents in the mycelia of *A. niger* and *Amylomyces rouxii*. Goupil considered mineral phosphorus in the mycelium to be the product of organic phosphorus degradation while Koch and Reed believed mineral phosphorus to be changed to lecithin and protein phosphorus through an intermediate water-soluble phosphorus fraction. A view similar to that of the latter authors was held by Vorbrodt (112) who assigned a transitory role to the acid-soluble organic phosphorus fraction in the development of *Aspergillus niger* and an essential role to mineral phosphorus in the mycelium, without indicating the nature of those roles. As is evident, these concepts deal only with the incorporation of phosphorus into ultimate mycelial structure and give no hint of the respiratory role that has since emerged in living yeasts, bacteria, and animal cells. In these latter developments, the acid-soluble phosphorus fraction including inorganic orthophosphate P, labile phosphorus including pyrophosphate P, and organic phosphorus such as hexosephosphates and their various degradation products have been demonstrated to form an integral part of carbohydrate dissimilatory processes occurring within the cells in a manner comparable if not identical to the phenomenon of phosphorylation obtained with artificial preparations.

A similar possibility arises for the fungi here studied, but can be determined only through identification of the acid-soluble phosphorus constituents and through a study of their interrelationships under conditions similar to those obtained with yeasts (18, 50, 55, 65, 66, 70, 86) and bacteria (5, 79, 116). The suggestion by Nord *et al.* (76, 77, 78) that glucose dissimilation by *Fusarium* spp. need not go by way of phosphorylation has been criticized (65) on the ground that consideration was given only to the phosphorus in the medium rather than that in the mycelium (102), to which also might be added the anaerobic conditions prevailing under the experiment with consequent phosphorus mineralization (67).

The attempt in the present work to determine the metabolic significance of phosphorus through following the changes in the acid-soluble phosphorus constituents in macerated mycelial preparations proved unsuccessful because phosphorus mineralization was the predominating if not the exclusive reaction. This same general behavior of phosphorus mineralization was obtained under addition of various substances such as glucose, NaF, toluene, KCN, ethyl urethane, iodoacetate and phlorhizin which have proven so successful in similar studies with blood cells (31), yeast, and bacteria. The conclusion may be drawn that conditions were

those of inhibited respiratory activity obtained through inadequate aeration.

The observed mineralization of phosphorus and changes in the acid-soluble phosphorus constituents perhaps finds its counterpart in a similar behavior of phosphorus on autolysis of other cells such as those of muscle (23), liver (2, 26, 90, 100), "magenmucosa," spleen, kidney, pancreas, heart and striated muscle (108), brain (1, 28), blood (89), yeast (61), and bacteria (116). Fungi have scarcely been investigated (33, 40). The increases by approximately 20 per cent of the acid-soluble phosphorus presumably had their origin in either or both the lipoid phosphorus fraction (69, 108) or the nuclein phosphorus (33, 40). Such increases were slightly greater and more rapid in the presence of toluene and lower and less rapid in the presence of NaF. Glucose enhanced these increases under both treatments. Inorganic orthophosphate showed ultimate increases of approximately 50–200 per cent which would indicate that by far the greatest mineralization of phosphorus occurred within the acid-soluble phosphorus fraction. The observed increases in amount of organic-phosphorus fractions equal to or smaller than the increases in total phosphorus from non-acid soluble phosphorus fraction would lend support to the concept that the latter fraction was mineralized directly and by way of conversion into some acid-soluble organic phosphorus compound. The identity of the latter with the organic phosphorus resistant to 3-hour acid hydrolysis is suggested by the observed initial increase in this fraction and its subsequent slow decrease during progressive autolysis. The comparative persistence of the labile phosphorus fraction and the more rapid and nearly complete disappearance of the 3-hour acid-hydrolyzable phosphorus fraction would suggest the intermediate role of the former in the dephosphorylation (and possible phosphorylation) of the latter (90).

The metabolic behavior (CO_2 evolution and O_2 consumption) of macerated mycelial preparations of *Chaetomium funicola* is in respect to endogenous activity analogous to that observed for yeast (98) and "*Fusarium* sp. H" (29). Exogenous activity is similar as to sensitivity to NaF and iodoacetate but different in its sensitivity to cyanide and in the R. Q. values which are characteristic for respiration. These findings and others[7] suggest that exogenous activity with macerated mycelial preparations of *Chaetomium funicola* is fermentative as well as respiratory, probably in accordance with the Pfeffer-Blackman hypothesis (110). Of interest in this connection are the recent views of Runnstrom, Borei and Sperber (91), and Borei (8) who considered the inhibitory effect of NaF to be concerned directly with the respiratory mechanism rather than with the interruption of a primary fermentation (the anaerobic phase of respiration).

[7] In culture experiments spores of *C. funicola* failed to germinate in a nutrient medium containing iodoacetate in concentrations of M/1,000 or greater while a 50 per cent reduction in growth occurred with M/10,000 iodoacetate. Sodium fluoride M/500 caused a 75 per cent reduction in growth, and higher concentrations of NaF caused further reductions in growth till complete inhibition was obtained with M/10 NaF.

The observed induction period and subsequent rise in rates of metabolic activity of macerated mycelial preparations of *C. funicola* suggest a partially disorganized system which is undergoing autolysis (74, 75).

The failure in the present study to isolate phosphoglyceric acid and to detect any significant decrease in inorganic orthophosphate concentration in the experimental medium cannot be taken as direct evidence against the concept of phosphorylation as a process occurring in the carbohydrate metabolism of *C. funicola* (19). The manner of treatment of the mycelium may have been too drastic and age of the mycelium may be an important consideration also (29, 32, 36, 102, 106). The demonstration of methylglyoxal, pyruvic acid, and acetaldehyde in the present work also cannot be considered evidence against the phosphorylation scheme but may even favor it since their origin may reside with some phosphorylated intermediate product (80).

In the present study recourse was made to the use of macerated mycelial preparations with the purpose of subdividing and macerating the mycelial hyphae to obtain a more uniform distribution of the material throughout the experimental medium. Although grinding with sand had destroyed some of the mycelial fragments, microscopic examination revealed the presence of considerable quantity of intact hyphae. Tamiya (104) used a similar preparation to determine the dehydrase activity of *Aspergillus oryzae*. Bernhauer and Wolf (6) used *A. niger* mycelium macerated in a mortar with pestle under addition of $CaCO_3$ and obtained the formation of gluconic and oxalic acids in the presence of added glucose. More recently Gould and Tytell (29) successfully used young hyphal materials of "*Fusarium* sp. H" that had been dispersed by shaking with glass beads in a phosphate buffer solution and likewise older mycelia that had been finely minced before shaking with glass beads for dispersion. Earlier investigators of mycelial respiratory activity used carefully teased-out mycelial hyphae (32), intact, uninjured mycelial mats (46, 47, 85, 102, 103), variously dried preparations (37, 48, 49), or press juice (42, 71).

Three species of *Fusarium* and *Aspergillus niger* were included with *Chaetomium funicola* in the present study to obtain a range of fungi differing essentially in their biochemical properties as revealed by the products accumulated. Thus, while *C. funicola* converts glucose mainly to carbon dioxide and mycelium, *Aspergillus niger* produces such acids as oxalic, citric, and gluconic; and *Fusarium* spp. are known to produce ethanol in fair amounts. The inclusion of *Aspergillus niger* was of interest because of its ability to form gluconic acid directly from glucose by the glucoseoxidase enzyme without the necessary participation of phosphorus, and because of the disputed (10) Chrzaszcz-Tiukow hypothesis (4, 14, 105) underlying the formation of oxalic, citric, and other acids from glucose, which assumes an initial anaerobic phase of glucose dissimilation identical to alcoholic fermentation. *Fusarium* spp. were included because the mechanism underlying their formation of alcohol has been assumed to be similar to that of yeasts (29) except that Nord (76, 77, 78) has suggested that glucose dissimilation by some *Fusarium* spp. need not go by way of phosphorylation.

SUMMARY

1. The phosphorus relations of *Chaetomium funicola* were studied with cultures developing on Czapek-Dox medium. The depletion of phosphorus in the medium was followed through inorganic orthophosphate P and total phosphorus analysis at several intervals in the development of the fungus. Analysis was made of the acid-soluble phosphorus constituents in the mycelium of *C. funicola* and four other fungi. An interpretation of the significance of these acid-soluble phosphorus constituents in the mycelium was sought in the light of the phenomenon of phosphorylation applied to carbohydrate dissimilation.

2. *Chaetomium funicola* continued to develop and remove phosphorus from the medium throughout the studied period of 23 days' development. Mycelial development was not stopped with depletion of the inorganic orthophosphate supply in the medium as obtained with one-fourth normal initial concentrations of KH_2PO_4 in the medium, but contined presumably in a similar manner to that which allowed for mycelial development in the media with no additions of KH_2PO_4.

3. Greater removal of phosphorus from the medium occurred with higher initial concentrations of phosphorus, yielding correspondingly higher total and percentage phosphorus contents in the mycelium.

4. Varying the initial pH of the medium did not influence the removal of phosphorus from the medium except under alkaline and very acid conditions where the amount of mycelium formed and phosphorus removed was retarded.

5. A non-orthophosphate P fraction in the medium was detected in amounts of 20 per cent of the total phosphorus remaining in the medium at 10 and 23 days' development of the fungus. Analysis showed this fraction to contain labile phosphorus and organic phosphorus of a type resistant to hydrolysis. Its presence was presumed due to its liberation from ageing or autolyzing hyphae.

6. Inorganic orthophosphate P, labile phosphorus, and organic phosphorus of the difficultly hydrolyzable type were found in the acid-soluble constituent of mycelia of *C. funicola, Aspergillus niger, Fusarium lini, F. bulbigenum* var. *niveum,* and *F. oxysporum* var. *cubense.*

7. Comparison of the acid-soluble phosphorus constituents in mycelium from 8- and 18-day-old cultures of *Chaetomium funicola* revealed a lower percentage of inorganic orthophosphate P and a higher percentage of labile phosphorus in the older mycelium. Indications were obtained in both mycelia that the organic phosphorus was composed of two parts, one resistant to acid hydrolysis and the other quite susceptible.

8. The significance of the acid-soluble phosphorus fractions in *C. funicola* and the other four fungi was considered in the light of recent investigations on carbohydrate metabolism with living yeasts, bacteria, and animal cells, and was investigated particularly with *C. funicola* for a similar interpretation.

9. Incubation of macerated mycelial preparations of *C. funicola* and *Fusarium* spp. at 30°C. with a view to determining the interrelationship

of the different acid-soluble phosphorus fractions in the mycelium in a manner similar to that followed for living yeasts, bacteria, and blood cells revealed autolytic mineralization of phosphorus to be the predominating reaction of such preparations under the conditions employed. Maximal increases of 10–27 per cent in total acid-soluble phosphorus and 50–200 per cent in inorganic orthophosphate P were obtained within a period of approximately 18 hours. Labile phosphorus and 3-hour acid-hydrolyzable organic phosphorus generally decreased in amount with progressive autolysis, with the decrease of the latter being more rapid and more complete than that of the former. Organic phosphorus resistant to 3-hour acid hydrolysis showed initial increases with subsequent decreases suggesting its origin to reside with some acid-insoluble fraction. Sodium fluoride M/30 retarded while toluene accelerated these changes.

10. A determination of the respiratory activity of macerated mycelial preparations of *C. funicola* in a Warburg-Barcroft respirometer revealed endogenous and exogenous activities of these preparations with the former activity being characteristically oxidative and the latter suggestive of respiration with an initial fermentative phase. Evidence obtained suggested that these preparations were partially disorganized and undergoing autolysis.

11. Direct attempts toward demonstrating phosphorylation as a significant process in carbohydrate metabolism of *C. funicola* using macerated mycelial preparations showed neither phosphoglyceric acid formation nor phosphorus uptake. Methylglyoxal, pyruvic acid, and acetaldehyde formation, however, was readily demonstrated.

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HYGROSCOPICITY, PROPERTIES IN BORIC ACID SOLUTION, AND SPECIFIC VISCOSITIES OF MIXTURES OF THE DIASTEREOISOMERIC 2,3-BUTANEDIOLS[1]

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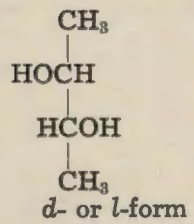
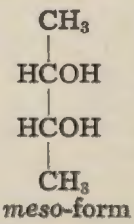
2,3-butanediol, commonly called 2,3-butylene glycol, has also been named as β-butylene glycol, symmetrical butylene glycol, 2,3-dihydroxybutane, β,γ-dihydroxybutane, symmetrical dimethyl ethylene glycol, and pseudo butylene glycol. The glycols are generally classified as aliphatic compounds whose formulas contain two hydroxyl groups. Aldehydes and ketones give derivatives, such as the acetals and the hemiacetals, containing two substituted hydroxyl groups, but such compounds are not classed as glycols.

The general properties and methods of preparation of the glycols have been discussed in detail by Lawrie (12). Over 100 glycols are known but, until recently, ethylene glycol and pinacol were the only ones to assume importance. The boiling points of the glycols range upward from 180° C., but there is no correlation between the boiling points and molecular weights. The glycols are very hygroscopic, and it is often difficult to remove the last traces of water from them. A summary of some of the most important properties of the glycols has been given by Lees (13).

The glycols of the general formula R·CHOH·CHOH·R exist in two diastereoisomeric forms. These forms for 2,3-butanediol are:



d- or *l*-form



meso-form

HISTORICAL

Harden and Walpole (9) were the first to show the production of acetylmethylcarbinol and 2,3-butylene glycol by the action of bacteria on sugars. They found that 27 per cent of the dextrose fermented by

[1] This work was supported by a grant from the Industrial Science Research Institute of the Iowa State College for studies on the fermentative utilization of agricultural products. The paper is based on the thesis presented in 1942 by Mr. T. M. Lees for the degree of Master of Science. Publication has been delayed by a War Department secrecy order forbidding publication of information in regard to *l*-2,3-butylene glycol. The secrecy order was lifted as of November 29, 1943.

B. lactis aerogenes, under anaerobic conditions, was converted to 2,3-butylene glycol. Fulmer, Christensen, and Kendall (7) determined optimum conditions for the production of 2,3-butylene glycol by the action of four species of *Aerobacter* on sucrose in a synthetic medium. While there were no great differences in glycol yields among the organisms tested, *Aerobacter aerogenes* was found to be the most satisfactory for general use. Under optimum conditions 98 per cent of the sugar was fermented of which 47 per cent was converted to 2,3-butylene glycol; this yield is about 90 per cent of theory. The authors also presented a brief review of previous work on the fermentative production of 2,3-butylene glycol.

Walpole (15) was one of the first to investigate the nature of the isomers present in fermentation 2,3-butylene glycol. He obtained, by fractionation, two phenylurethane derivatives melting at 199.5° and 157° C., respectively. The former constituted about 90 per cent of the glycol. While he did not commit himself as to the nature of the predominating compound, later information indicates that it was the *meso*-glycol. Böeseken and Cohen (3) decided, on the basis of resolution data, that the *meso*- form predominates in fermentation 2,3-butylene glycol.

The best method heretofore available for the analysis of mixtures of the *meso*- and *dl*-glycol is based upon melting point data presented by Wilson and Lucas (17); the glycols were prepared synthetically. They gave the melting points of the *meso*- and *dl*-glycols as 34.4° and 7.6°, respectively. The melting point-composition curve shows an eutectic point, by extrapolation, at about —15° C. and about 40 per cent of *meso*-glycol content. The melting points of mixtures containing less than 80 per cent of the *meso*- form are difficult to determine. The hygroscopicity of the glycol, its tendency to form supercooled solutions, and the increased viscosity at low temperatures all contribute to making the melting point difficult to determine near the eutectic point. It should also be noted that, in the temperature range of 7.6° to —15° C., the melting point is a two-valued function of the composition, that is, there are two combinations of the glycols with identical melting points.

There is need for the development of more adequate methods for the determination of the proportions of the glycols in mixtures. The data presented in this paper involve mixtures of the *meso*- and *l*-2,3-butylene glycols. The *d*-2,3-butylene glycol should have the same physical constants and behavior as the *l*-form except as to sign of optical rotation.

MATERIALS

The 2,3-butylene glycol used as the source of the *meso*-glycol employed in obtaining the data presented below was obtained by the action of *Aerobacter aerogenes* upon dextrose. The melting point showed the glycol to contain 90 per cent of the *meso*- form. Fulmer, Underkofler, and Bantz (8) studied the production of acetylmethylcarbinol by the action of *Acetobacter suboxydans* upon the 2,3-butylene glycol described above. The yield of carbinol was about 90 per cent of theory. The unfermented

glycol was recovered and purified. The angle of rotation was $[\alpha]_D^{25}+10.15$. It was concluded from these studies that the organism attacked the *meso*-glycol preferentially and that the original glycol consisted of *meso*- and *d*-glycol with little or no *l*-glycol.

The *meso*-glycol used in subsequent experiments was prepared as follows. The fermentation glycol, containing 90 per cent of the *meso*-form, was subjected to vacuum distillation through a Widmer column with an inner core 16 inches in length. Fractions were removed by means of an all-glass fraction cutter. The pressure was from 1 to 2 mm. of mercury; the rate of distillation was approximately 1 drop each 4 seconds. The fraction used for further purification boiled at 180° C. (729.2 mm.).

Fractional crystallization, using isopropyl ether as the solvent (17), was employed for isolation of the *meso*-2,3-butylene glycol. About 400 ml. of the fraction, prepared as above described, was dissolved in 600 ml. of freshly distilled isopropyl ether. The all-glass apparatus consisted of a 2-liter, long-necked boiling flask closed with a calcium chloride tube. The mixture was cooled in an ice bath and left for half an hour with occasional shaking. The ether was then carefully decanted from the porous mass of crystalline *meso*-glycol. Another portion of 600 ml. of isopropyl ether was added and the crystallization repeated. In all, six recrystallizations were made. The flask containing the last fraction was evacuated over night. The glycol was then melted and quickly transferred to a round-bottomed flask and subjected to distillation through an insulated Vigreaux column. After the ether had distilled, the glycol came over at 181° C. The distillate was redistilled through the same column, about 100 g. of the final distillate being obtained. The melting point determination showed this final fraction to contain at least 99 per cent of the *meso*-glycol. The angle of rotation was zero.

The *l*-2,3-butylene glycol was obtained through the courtesy of Dr. R. D. Coghill of the Northern Regional Research Laboratory. The material was redistilled through an insulated Vigreaux column. The angle of rotation was $[\alpha]_D^{25}-13.0$. In absence of evidence to the contrary, this glycol was assumed to be the pure *l*-2,3-butylene glycol. If this assumption should later prove to be incorrect some of the results given below would need to be recalculated, but the basic principles established would still hold.

Each type of glycol was dispensed from a separate 50 ml. burette equipped with a calcium chloride tube at the top and a removable calcium chloride tube at the tip.

EXPERIMENTAL

1. *The Hygroscopicity of 2,3-Butylene Glycol*

It was previously noted that the glycols are very hygroscopic. Data are given in Table 1 showing the hygroscopicity of 2,3-butylene glycol. The glycol used, produced by the action of *Aerobacter aerogenes*, was the same material as that used for the separation of the *meso*-glycol. A 17.7 g.

sample of the glycol, in a crystallizing dish, was placed in a desiccator containing water and left at room temperature which did not vary greatly from 25° C. The depth of the glycol was about 1 cm. and the surface-volume ratio was 1.07. The dish and contents were weighed periodically

TABLE 1

HYGROSCOPICITY OF 2,3-BUTYLENE GLYCOL

Time, Hours	W*		Time, Hours	W*	
	Exper.	Calc. from Eq. (1)		Exper.	Calc. from Eq. (1)
4	3.2	4.0	263	62.0	61.7
16	10.1	10.0	306	69.1	68.1
29	14.3	14.7	342	74.7	73.1
41	18.6	18.4	380	79.9	78.3
53	21.8	21.7	428	86.4	84.7
66	25.3	25.1	474	92.4	90.6
90	30.8	30.8	534	99.4	98.0
116	35.7	36.3	602	106.8	105.7
163	44.4	45.2	672	113.3	113.8
190	49.5	50.0	784	125.6	125.3

* W = grams of water absorbed per 100 grams of glycol.

to determine the weight of water absorbed. The percentage of water absorbed (grams of water absorbed per 100 grams of glycol), w, proved to be a parabolic function of the time, t, the equation being:

$$(1) \quad \log w = 0.651 \log t + 0.216.$$

Calculated values, using this equation, are given in Table 1.

These data emphasize the importance of care in keeping moisture from glycol samples. The occasional abnormally large deviation of experimental values from the theoretical, shown by data in subsequent experimental results, may be attributed in large measure to accidental entrance of moisture into the prepared samples of the glycols.

2. *The Specific Viscosities of Mixtures of Meso-2,3-Butanediol and l-2,3-Butanediol*

Data in Table 2 show the specific viscosities of mixtures of the two glycols. The apparatus consisted simply of an ordinary 5 ml. pipette with an added graduation below the bulb. The determinations were made at 30° C. The time required for the mixture to flow from one graduation to another was measured by means of a stop-watch. The ratio of the drainage times for the mixtures to that for water is the specific viscosity. The densities of the two glycols are so near to each other and to that of water that this simple relation is justified.

The specific viscosity, s, proved to be an exponential function of the

percentage of *meso*-glycol of the type $\log(y - c) = mx + b$, from which was derived the equation,

$$(2) \quad \% \text{ meso-glycol} = 140.0 \log(s - 2.87) - 55.2.$$

Values calculated by this equation are given in Table 2.

The specific viscosity method is simple and satisfactory for the determination of mixtures of the glycols. However, it should again be empha-

TABLE 2

SPECIFIC VISCOSITIES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

		Per Cent *meso*-Glycol	
t^* ..	s†	Experimental	Calc. from Eq. (2)
44.3	5.34	0	0
48.7	5.88	16.6	11.8
58.6	7.07	33.8	32.0
70.6	8.52	50.1	50.1
85.5	10.32	66.9	66.9
106.4	12.83	83.4	84.5
130.4	15.72	100.0	100.0
8.3 (water)			

* s = specific viscosity.
† t = drainage time in seconds.

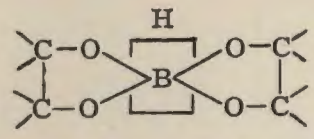
sized that extreme care must be taken to insure against taking up of moisture by the samples.

3. *Specific Conductivities and pH Values of Mixtures of Meso-2,3-Butanediol and l-2,3-Butanediol*

It is well known that certain polyhydric compounds react with boric acid, in solution, to form complex acids which are strong electrolytes. For example, boric acid can be titrated as a monobasic acid in the presence of glycerol or mannitol using phenolphthalein as indicator. Magnanini (14) was one of the first to apply conductometric methods to polyhydric compounds in boric acid solution. Böeseken and coworkers (1, 2, 3, 4, 5, 6) extended the earlier work with a primary interest in the stereochemical relations of the polyhydric compounds to the phenomenon. The methods developed by them have proved useful in determining the configurations of certain of the sugars.

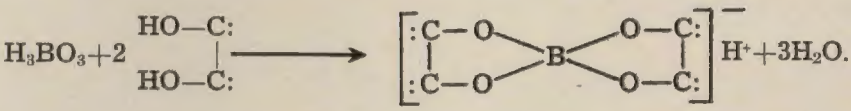
The increase in electrical conductivity, with accompanying decrease in pH, of the polyhydroxy compound in boric acid solution is associated with the formation of a more highly ionizable complex of the two compounds. Conditions are most favorable to the formation of the complex when the two hydroxyl groups are on adjacent carbon atoms and lie on the same side of the carbon atoms. Hermans (10) assigned the follow-

ing structure to the complex:



That is, two molecules of the polyhydroxy compound combine with one molecule of the boric acid.

Böeseken (6) confirmed the results of Hermans and wrote the reaction as follows:



Whitmore (16) described the complex as follows: "The complex has the tetrahedral grouping of five atoms and 32 electrons which characterizes the ions of H_2SO_4, H_3PO_4, and $HClO_4$. Similar products are formed with arsenious acid. Whenever two hydroxyls on adjacent carbons can form this tetrahedral arrangement with a boron or an arsenic atom the conductivity is increased. The only thing which can prevent this union is the location of the two adjacent hydroxyls on opposite sides of the plane of a ring compound such as the furanose and pyranose forms of sugars." It may well be that with higher glycols hindered rotation about a single bond will have the same effect.

Böeseken and Cohen (3) have studied the configurations of the 2,3-butylene glycols by the boric acid method. They used a glycol obtained by the fermentative action of *Aerobacter aerogenes* and a *dl*-glycol obtained by synthesis. They concluded that the hydroxyl groups of both samples of glycol were in an unfavorable position to form the boric acid complex.

Previous reports in the literature on this subject are so meager and inconclusive that further work seemed desirable. The authors present data in the present communication which show that the *meso*- and *l*-2,3-butylene glycols do differ in respect to properties in boric acid solution to a sufficient degree to form the basis for analytical methods for the determination of the composition of mixtures of the glycols.

The conductivity apparatus was a simple setup employing a Leeds and Northrup Student Potentiometer, two decade boxes, a 1,000-cycle audio-oscillator, and head phones. The cell used was of the Freas type with platinized electrodes. All determinations were made at 25° C. The specific conductivity was calculated by the relation $L = K/R$, in which L is the specific conductivity, K is the cell constant and R is the resistance of the solution in ohms. The pH values were determined with a Cameron glass electrode apparatus.

a. Specific conductivities. Preliminary experiments were made varying the glycol and boric acids separately to determine the best conditions for the standardization of the method. In the standard conditions chosen, each sample contained 5.00 g. of the mixture of glycols diluted to 50.0 ml. with 0.5 M boric acid solution. A maximum conductivity was obtained with 9.00 g. of glycol mixture in 0.5 M boric acid solution. However, the supply of glycol was limited, and the difference between the conductivities with 5.00 g. and 9.00 g. of glycol was not sufficient to warrant using the larger amount. The conductivities and pH values were determined after the mixtures had been allowed to stand for 48 hours to assure stable conductivity values.

The specific conductivity data are given in Table 3. It is evident that the specific conductivity decreases markedly with increase in content of

TABLE 3

SPECIFIC CONDUCTIVITIES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

	Per Cent *meso*-Glycol	
$^*L \times 10^5$	Experimental	Calc. from Eq. (3)
6.91	0	−0.6
6.13	16.6	16.6
5.28	33.8	35.6
4.61	50.1	50.6
3.88	66.9	67.0
3.13	83.4	83.7
2.49	100.0	98.1

* L = specific conductivity.

meso-glycol. The specific conductivity is a linear function of the composition, the equation being:

$$(3) \quad \% \text{ meso-glycol} = 153.6 - (22.3 \times L \times 10^5).$$

Calculated values, using this equation, are given in Table 3. The specific conductivity of the standard fermentation glycol was 2.96×10^5. The calculated percentage of *meso*-glycol, using equation (3), was 88 per cent. This value agrees with that of 90 per cent obtained by the melting point method.

b. pH values. The pH values of the glycol mixtures, in 0.5 M boric acid solution, are given in Table 4. As would be expected from the conductivity data, the pH increased with increased content of the *meso*-glycol. A mathematical analysis of the data showed the hydrogen ion activity to be a linear function of the composition. The hydrogen ion activity is defined as $a_{H^+} = \text{antilog } (-\text{pH})$. From this relation the following equation was derived:

$$(4) \quad \% \text{ meso-glycol} = 123.6 - (4.81 \times a_{H^+} \times 10^5).$$

Calculations using this equation are given in Table 4.

It is evident that the change in pH values is not as wide as the change in specific conductivity. The former offers the advantage of general availability of equipment for measuring pH. While traces of water

TABLE 4

pH VALUES OF MIXTURES OF *meso*-2,3-BUTANEDIOL AND *l*-2,3-BUTANEDIOL

pH	Per Cent *meso*-Glycol	
	Experimental	Calc. from Eq. (4)
3.59	0	0
3.65	16.6	15.9
3.71	33.8	29.8
3.82	50.1	51.0
3.93	66.9	66.8
4.08	83.4	83.6
4.31	100.0	100.0

are not as serious in these determinations as with specific viscosity determinations, small traces of ionized materials should be carefully avoided.

SUMMARY

1. Detailed procedures have been described for the isolation of *meso*-2,3-butylene glycol from the glycol produced by the action of *Aerobacter aerogenes* upon sugars.

2. Data are presented on the hygroscopicity of 2,3-butylene glycol. The glycol proved to be highly hygroscopic. The water absorbed per 100 g. of glycol, in an atmosphere saturated with water vapor at about 25° C., is a parabolic function of the time of exposure.

3. The specific viscosity of mixtures of *meso*- and *l*-2,3-butylene glycols is an exponential function of the composition of the type $\log (y-c) = mx + b$. The method developed furnishes a simple and satisfactory procedure for the analysis of mixtures of the glycols.

4. The specific conductivity of mixtures of *meso*- and *l*-2,3-butylene glycols in 0.5 M boric acid solutions is a linear function of the composition. The hydrogen ion activity of the mixtures is a linear function of the composition. There is a wider range of change in conductivities than the change of pH values. Both conductivities and pH values in boric acid solutions furnish procedures for the analysis of mixtures of the two glycols.

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