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SEASONAL FOOD PREFERENCE TRENDS OF EASTERN RUFFED GROUSE IN IOWA AS SHOWN BY DROPPING ANALYSIS[1]

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Short articles have appeared from time to time in various ornithological periodicals regarding the food habits of the ruffed grouse. Most of them have dealt with direct observations, and stomach and crop analysis.

From August, 1938, until March, 1940, the writer spent considerable time in the field investigating the cover requirements of the ruffed grouse (*Bonasa umbellus umbellus*) in northeastern Iowa. Incidental to the cover research, droppings were collected and analyzed for food material content. Assistance and guidance in this research was given by Dr. G. O. Hendrickson, Department of Zoology and Entomology, and Mr. Thomas G. Scott, United States Fish and Wildlife Service. Identification of insect remains was facilitated by the assistance of Dr. H. H. Knight, Department of Zoology and Entomology.

Droppings were found at night roosts and day time loafing roosts which were located by searching under hazelnut brush and dogwood brush in the vicinity of scratch beds and dust baths. Night roosts contained from 30 to 70 individual fecal pellets to the roost sample. Loafing roosts contained from 10 to 15 pellets. Small samples containing less than 10 pellets were not collected.

Collected fecal material was kept separately wrapped in small sacks. Preparatory to examination the pellets were soaked in warm water until they could easily be broken down by passing tap water over them. A number 40 sieve was used in the separation process. Ureates, fine matter, and solubles passed through the sieve and left larger fragments that could be identified by comparison with a control collection with the aid of a 10-power binocular microscope. Before washing through the sieve the pellets were examined for the presence of anthers and other small materials easily washed through the sieve.

A total of 176 samples was examined. The numbers of samples for the seasons were as follows: 33 spring, 26 summer, 51 fall, and 66 winter. Each individual pellet in a sample was examined; each food item found in a pellet was given the value of one frequency, and percentages were derived from the sums of the frequencies of all food items in the sample. For the entire season, percentages were derived from the cumulative percentages of all samples examined. These figures may not give the

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true percentages of volume or weight of foods taken, and soft-bodied animals or finely digested food matter that is easily destroyed in digestion may not be represented in this analysis as they would be in crop analysis and stomach analysis procedures. The chief value of this method is in showing seasonal trends of food habits among a game species too scarce to be killed for stomach and crop analysis.

The seasonal food trends in order of percentage of seasonal frequency are shown in Table 1. Percentages of animal foods taken in each of the

TABLE 1

IOWA RUFFED GROUSE FOODS IN ORDER OF SEASONAL FREQUENCY

	Percentage
Spring foods	
Miscellaneous buds	30.5
Hog peanut leaves (*Amphicarpa monoica*)	11.1
Grass leaves (Gramineae)	9.3
Hazelnut catkins (*Corylus americana*)	9.1
Choke cherry buds (*Prunus virginiana*)	6.3
Dandelion leaves (*Taraxacum*)	6.0
Strawberry leaves (*Fragaria*)	5.1
Birch buds (*Betula lutea*)	4.1
Strawberry fruit	3.5
Miterwort leaves (*Mitella diphylla*)	3.1
Windflower leaves (*Anemone*)	2.2
Oak buds (*Quercus*)	2.1
Elm seed (*Ulmus*)	1.4
Bees (Apidae)	1.3
Maple seeds (*Acer*)	.5
Sweet Cicely seeds (*Osmorhiza claytoni*)	1.1
Spiders (Araneida)	.9
Grasshoppers (Acrididae)	.8
Raspberry leaves (*Rubus*)	.8
Ichneumon fly (Ichneumonidae)	.4
Saxifrage leaves (*Sullivantia Sullivantii*)	.3
Moss (Musci)	.2
Summer foods	
Beetles (Carabidae)	10.0
Bluegrass leaves (*Poa*)	9.1
Miscellaneous leaves	8.0
Beetle (Curculionidae)	7.4
Acorn (*Quercus*)	7.2
Tree cricket (*Pterophylla camellifolia*)	6.8
Gravel	5.5
Raspberry fruit	4.0
Bug (Pentatomidae)	3.7
Undetermined beetles (Coleoptera)	3.2
Dogwood seeds (*Cornus*)	2.8
Sandcherry seeds (*Prunus pumila*)	2.6
Cockroach (*Parcoblatta pennsylvanica*)	2.6
Trefoil leaves (*Desmodium*)	2.4
Miscellaneous buds	2.2
Ants (Formicidae)	2.1
Beetles (Lucanidae)	2.1
Beetles (Chrysomelidae)	1.2
Sedge seeds (*Carex*)	1.1

	Percentage
Crickets (*Gryllus*)	1.1
Grasshoppers	1.1
Spider	1.0
Pigeon grass seeds (*Setaria*)	1.0
Blueberry fruits (*Vaccinium canadense*)	1.0
Ichneumon fly (Ichneumonidae)	.9
Undetermined Hymenoptera	.8
Beetle larvae (Coleoptera)	.7
Beetles (Tenebrionidae)	.7
Sweet Cicely seeds	.7
Bluegrass seeds (*Poa*)	.6
Hazelnut fruit (*Corylus americana*)	.5
Wasps (Chalcidae)	.5
Wasps (Braconidae)	.5
Crane flies (Tipulidae)	.5
Poplar buds (*Populus*)	.5
Beetles (Platystomidae)	.4
Self-heal leaves (*Prunella vulgaris*)	.4
Lace bugs (Tingitidae)	.4
Beetles (Scarabaeidae)	.4
Fly larvae (Diptera)	.4
Beetles (Otiorhynchinae)	.4
Harvestmen (Phalangidae)	.3
Choke cherry fruit	.3
Honeysuckle fruit (*Lonicera*)	.3
Nine-bark seeds (*Physocarpus*)	.2
Pigweed seeds (*Amaranthus spinosus*)	.2
Poison ivy seeds (*Rhus toxicodendron*)	.2
Solomon's seal seeds (*Polygonatum commutatum*)	.1
Beetles (Coccinelidae)	.1
Mouse bones and hair (*Peromyscus*)	.1
Feathers (Aves)	.1
Fall foods	
Acorn	21.5
Miscellaneous buds	11.9
Hazel catkins	10.9
Miscellaneous leaves	7.8
Sumac seeds (*Rhus*)	7.5
Sandcherry fruit	5.9
Gravel	4.0
Tree crickets	3.7
Hazelnut buds	3.4
Poplar buds	2.8
Bluegrass	2.5
Oak buds	2.0
Birch buds	2.0
Chrysomelid beetles	1.3
Dandelion leaves	1.2
New Jersey tea seeds (*Ceanothus*)	1.2
Mammal hair (Mammalia)	1.1
Grape fruits (*Vitis*)	1.0
Dogwood seeds	1.0
Honeysuckle seeds	1.0
Feathers	1.0
Undetermined bug (*Hemiptera*)	.8
Sedge seeds	.5
Carabid beetles	.5

	Percentage
Clover leaves (*Trifolium repens*)	.4
Red Haw fruits (*Crataegus*)	.4
Bugs (Miridae)	.4
Self-heal leaves	.3
Polygonum seeds (*Polygonum convolvulus*)	.3
Nine-bark seeds	.3
Ichneumon fly	.2
Undetermined beetles	.2
Mites (*Acarina*)	.2
Spiders	.2
Lead plant seeds (*Amorpha canescens*)	.2
Hog peanut seeds	.2
Trefoil seeds	.2
Winter foods	
Miscellaneous buds	23.0
Smooth sumac seeds (*Rhus glabra*)	20.7
Acorn	13.4
Pine needles	11.4
Staghorn sumac seeds (*Rhus typhina*)	8.5
Birch catkins	7.1
Poison ivy seeds	6.3
Miterwort leaves	3.4
Poplar buds	3.1
Oak buds	1.1
Miscellaneous leaves	.7
Honeysuckle seeds	.7
Gravel	.5
Lamb's-quarters seeds (*Chenopodium album*)	.1

seasons are as follows: winter, 0.0; spring, 3.4; summer, 49.4; and fall, 9.6. With the exception of small quantities of mouse hair and bones (*Peromyscus*), and feathers, possibly taken by preening, the entire animal food content consisted of arthropods of the orders *Arachnida* and *Insecta*. The high content of animal matter in the summer samples (July, August, and September) probably bears some relationship to samples from young birds whose droppings could not be differentiated from those of adults in August and September.

Gravel was very little in evidence in the winter and spring, but quite prevalent in the summer and fall samples. Gravel appeared in larger amounts when animal matter was present in the droppings. Winter

TABLE 2

SEASONAL VARIATIONS IN MAJOR FOOD GROUP CONSUMPTION IN PERCENTAGE

Fod Groups	Spring	Summer	Fall	Winter	Entire Year
Buds	43.0	2.7	22.1	27.2	23.8
Leaves	38.1	19.8	12.2	15.5	21.4
Seeds and fruits	6.4	22.6	41.2	49.7	29.9
Catkins	9.1	0.0	10.9	7.1	6.8
Animal matter	3.4	49.4	9.6	0.0	15.6
Gravel	0.0	5.5	4.0	0.5	2.5

and spring droppings contained many twig fragments taken with buds. December, January, February, and March samples contained sumac seeds and acorn fragments that may have acted as masticating agents in the place of gravel. Percentages of plant foods taken were as follows: fall 86.4, winter 99.5, spring 96.6, and summer 45.1. Major groups of plant items such as buds, catkins, leaves, and fruits are shown in Table 2.

Table 2 demonstrates that seeds and fruits are especially prominent in the grouse diet during the fall and winter months (October 1 to March 31). The prominent seeds and fruits of fall and winter are acorns and sumac seeds. Leaves occur in greatest abundance in spring and summer. Dominant as leaf foods during these seasons were hog peanut, bluegrass, dandelion, and wild strawberry leaves. The highest frequency of buds, contrary to expectations, occurred in the spring months with 36 per cent of the total of 43 per cent taken during April when the buds were beginning to swell and open.

THE DISSIMILATION OF GLUCOSE BY CHAETOMIUM FUNICOLA CKE.

II. Influence of Some Modifications in the Composition of Czapek-Dox Medium on the Rate of Glucose Dissimilation.[1]

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INTRODUCTION

In a previous study (4) *Chaetomium funicola* Cke. was found to dissimilate glucose slowly in Czapek-Dox medium under conditions of gentle or vigorous agitation of the medium. To determine the influence of the composition of this medium on the rate of glucose dissimilation, the concentrations of glucose and KH_2PO_4, the initial pH, and the source of inorganic nitrogen were studied. The general fate of the dissimilated glucose was followed through yields of mycelium and the pH changes in the medium. The results of these studies are presented.

The cultural behavior of *C. funicola* has not been previously reported. Futhermore, other species of Chaetomium have been scarcely investigated. Tschudy (8) found nine species of Chaetomium to develop on an agar medium with pH 4.2 to 11.0, with the best development occurring on the alkaline side and no growth at pH 3.0, while Dickson (1) reported the development of seven species in a linear manner on an agar medium containing different concentrations of glucose, starch, and potassium phosphate, with no formation of staling products.

EXPERIMENTAL

MATERIALS AND METHODS

The same culture of *C. funicola* was used here as in the previous study (4). Except for changes in concentration of glucose and certain inorganic salts, which will be indicated under each experiment, the composition of Czapek-Dox medium was as follows: 1 liter distilled water, 50.0 gm. Pfanstiehl's technical glucose, 2.0 gm. $NaNO_3$, 1.0 gm. KH_2PO_4, 0.5 gm. KCL, 0.5 gm. $MgSO_4 . 7H_2O$ and 0.01 gm. $FeSO_4 . 7H_2O$. Pyrex Erlenmeyer flasks of 250 cc. capacity were used as culture flasks. Forty cc. of liquid medium were introduced into each flask in the first and fourth experiments, and 35 cc. were introduced in the second and third. To avoid any expected change in the medium during sterilization

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at 15 lbs. steam pressure for 15 minutes, certain components of the medium were sterilized separately. With this procedure different components of the medium were prepared of such concentration that when combined with the major portion of the medium after sterilization and after the aseptic addition of a definite volume of ascospore suspension (usually 5 cc.), the final desired concentration of ingredients was obtained.

Non-absorbent cotton plugs served as stoppers for the culture flasks. Culture flasks were maintained on flat horizontal surfaces. The temperature ranged from 25° to 30°C. Cultures were decanted and filtered by suction through washed, dried, and weighed pieces of percale cloth. The mycelium was washed several times with hot distilled water in the culture flask and on the filter. Drying of the mycelium to constant weight was effected at approximately 70°C. for several days followed by maintenance over concentrated H_2SO_4 in a water-pump evacuated desiccator. The filtrates were made to 200 cc. volume and analyzed immediately.

Residual glucose in the filtrates was determined by the Bertrand modification of the Munson-Walker method (3) in experiments 1 and 4, and in experiments 2 and 3 by the Shaffer-Hartmann copper-iodate method as first modified and adapted to a semi-micro scale by Somogyi (6) and subsequently further modified by Schaffer and Somogyi (5), Harding and Downs (2), and Van der Plank (9). The solutions were prepared according to Van der Plank with the exception of the standard sodium thiosulphate solution, which was used as 0.005 N. This latter solution was prepared as recommended by Stiles, Peterson, and Fred (7). The starch solution was prepared as suggested by Willard and Furman (10) and the necessary precautions observed. Glucose values corresponding to the quantity of reduced $Na_2S_2O_3$ were read from an experimentally determined linear relationship. Hydrogen-ion concentration readings were made on the diluted filtrates with a Coleman glass electrode apparatus.

RESULTS

VARYING THE INITIAL GLUCOSE CONCENTRATION OF THE MEDIUM

Cultures were prepared without separate sterilization of the ingredients. The means of analyses of duplicate cultures after different periods of development are contained in Table 1 and are presented graphically in Figures 1, 2, and 3. The following observations were made from these data: (1) Glucose disappearance occurred in an approximately linear manner (Fig. 1). (2) The rate of glucose disappearance was greater at the higher concentrations of glucose than at the lower. (3) The disappearance of glucose from the medium when present in approximately 2.8 per cent initial concentration was nearly complete on the twenty-sixth day of fungus development. (4) Increases in mycelial weight on different initial concentrations of glucose were approximately the same for the first 17 days of fungus development. After this time further increases in

TABLE 1

EFFECT OF CONCENTRATION OF GLUCOSE IN CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	INITIAL PERCENTAGE GLUCOSE CONCENTRATION IN MEDIUM				
	2.8	5.5	7.5	10.1	13.5
	MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM				
0	28.7	55.2	75.5	101.2	135.6
7	24.4	47.4	75.0	92.2	127.4
17	5.1	23.3	50.3	72.1	95.6
26	0.3	20.5	42.4	59.1	85.8
47	0	0	13.5	34.5	49.3
	PH OF CULTURE MEDIUM				
0	4.72	4.95	4.58	4.77	4.61
7	6.29	6.20	6.10	6.22	6.35
17	7.87	7.52	7.63	7.71	7.84
26	7.62	7.15	7.10	7.46	7.35
47	8.70	8.04	6.46	6.25	6.28
	MYCELIUM FORMED, MILLIGRAMS				
7	105.8	130.6	75.1	120.5	141.9
17	339.1	332.6	378.9	326.8	327.5
26	347.5	409.0	428.6	649.2	568.2
47	279.4	620.0	753.7	752.2	945.7

mycelial weight occurred only in cultures containing initial glucose concentrations of approximately 5.5 per cent and greater (Fig. 2). (5) Autolysis of the mycelium occurred in cultures initially containing approximately 2.8 per cent glucose but only when the glucose content in the medium was reduced to a low level. (6) Progressive change of pH toward the alkaline side occurred during the initial 17 days of fungus development at all concentrations of glucose used, followed by a decrease (Fig. 3). This decrease was temporary and small in cultures initially containing approximately 2.8 and 5.5 per cent glucose but was prolonged and greater in cultures containing higher concentrations of glucose. The resumed rise in pH following the temporary decrease in the cultures containing the lower concentrations of glucose was attributed to the autolytic processes taking place in these cultures.

VARYING THE INITIAL pH OF THE MEDIUM

Cultures containing nearly 10 per cent glucose were prepared. Calculated quantities of separately sterilized hydrochloric acid and sodium hydroxide solutions were added to the sterile medium. The means of analyses of duplicate cultures (Table 2) showed that the fungus failed to grow at an initial pH of 2.12 and grew only slightly when the initial pH was 2.90. Considerably more growth of *C. funicola* occurred at higher initial pH values. Reduced growth was encountered again at pH 8.68,

TABLE 2

EFFECT OF INITIAL HYDROGEN-ION CONCENTRATION OF CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	INITIAL pH CULTURE MEDIUM								
	2.12	2.90	4.03	4.98	6.05	7.20	7.78	8.15	8.68
	MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM								
0	87.8	88.2	86.8	87.3	90.0	87.8	87.6	88.5	87.1
10	87.76	85.26	73.71	77.26	77.20	76.74	84.72	84.28	85.37
23	87.88	86.62	47.60	62.17	53.31	35.60	65.77	52.11	69.83
	pH OF CULTURE MEDIUM								
10	2.50	3.25	7.01	6.92	6.89	6.98	7.02	7.41	7.47
23	2.55	4.45	7.28	6.86	7.34	6.78	7.97	8.05	8.15
	MYCELIUM FORMED, MILLIGRAMS								
10	0.0	27.5	211.8	195.8	183.1	173.8	99.8	45.9	16.3
23	0.0	33.0	410.7	270.6	355.9	521.5	208.6	383.6	180.2

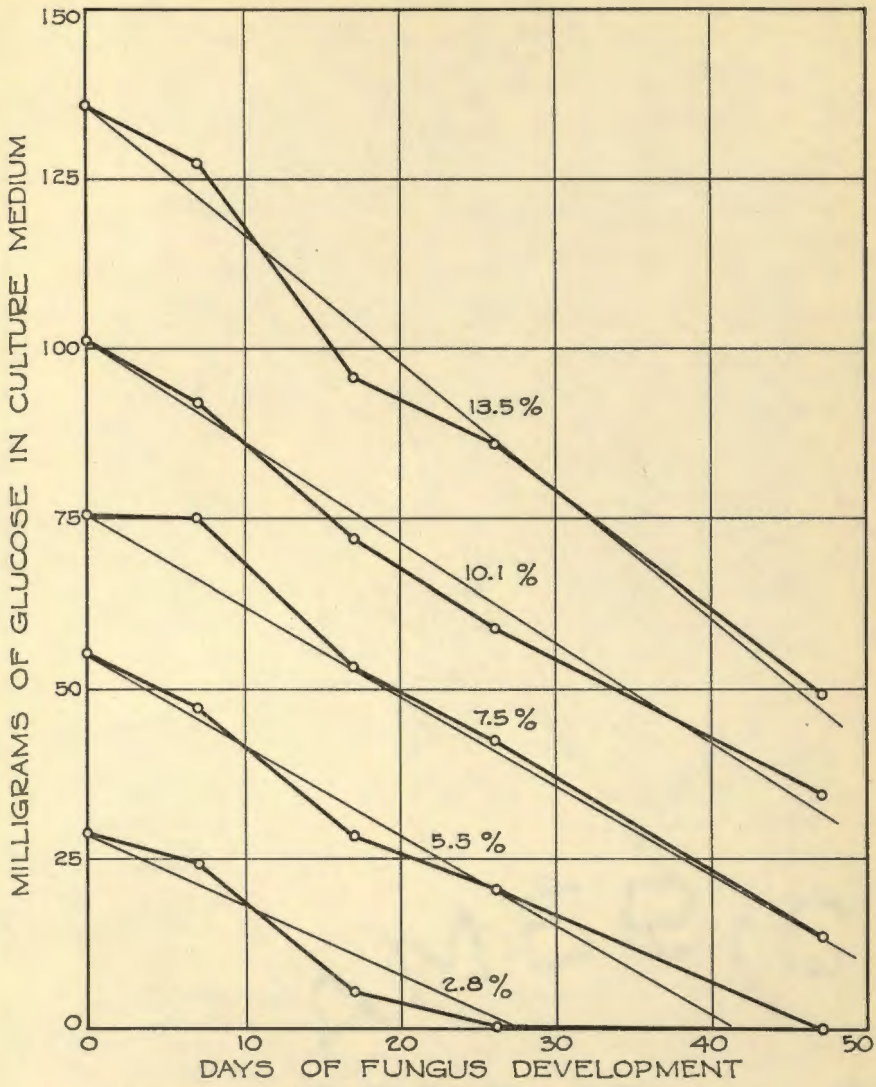

FIG. 1. Glucose remaining in Czapek-Dox medium containing different initial glucose concentrations after development of *Chaetomium funicola*.

which was the highest initial pH value tried. Tschudy (8) found pH 11.0 was the upper limit for growth of many species of Chaetomium, but in this test *C. funicola* was not included. He also found pH 3.0 was the lower limit for growth and the alkaline side yielded the optimum growth of these fungi. In the present experiment optimum growth of *C. funicola* after 10 days of development occurred at the initial pH value of 4.03, while after 23 days of development, optimum growth occurred at the initial pH of 7.20. In this latter period of development, however, three

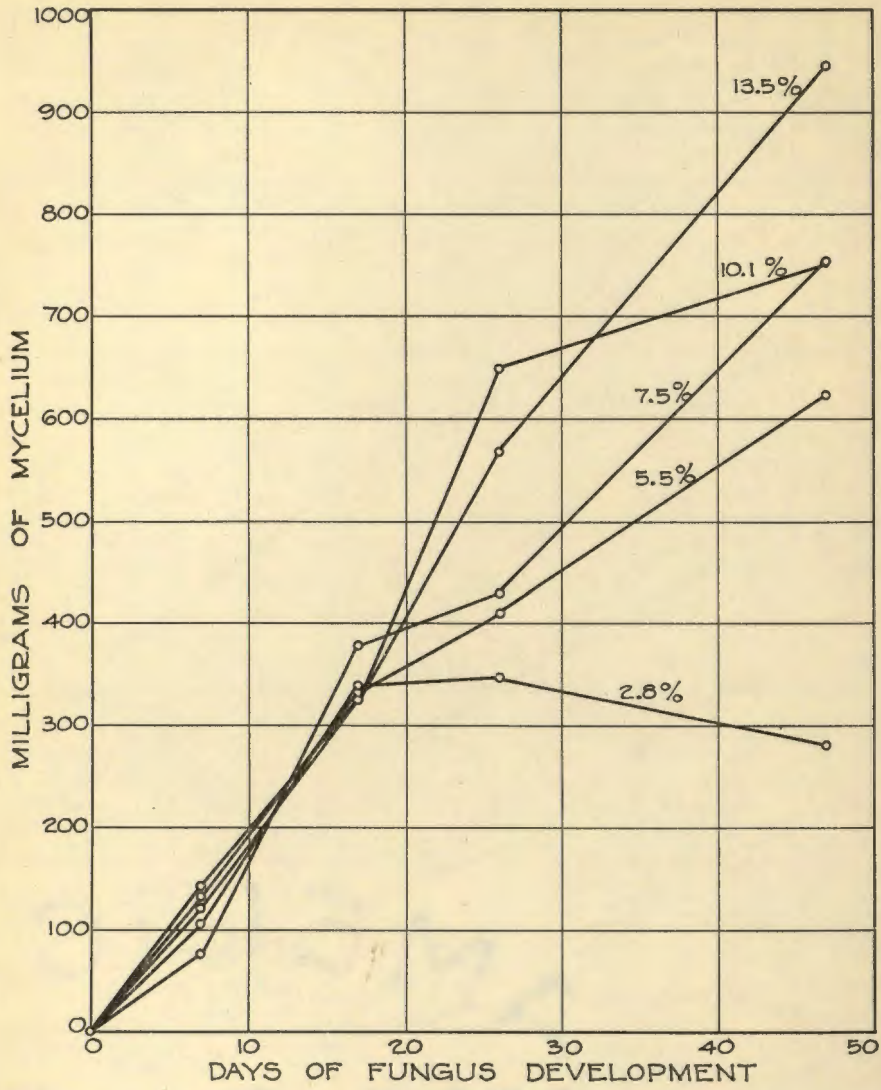

FIG. 2. Weight of *Chaetomium funicola* mycelium produced on Czapek-Dox medium containing different initial concentrations of glucose.

optimal initial pH values appeared, namely, 4.03, 7.20, and 8.15, with the latter value being the lowest of the three. The relation of change of pH of the medium to maximum yield of mycelium suggested the optimum pH for development of the fungus to be near neutrality and slightly on the alkaline side. The shift in pH of all cultures was toward neutrality and the alkaline side. The rate and amount of glucose dissimilation was closely associated with mycelium formation.

VARYING THE INORGANIC ORTHO-PHOSPHORUS CONCENTRATION OF THE MEDIUM

Cultures containing 10 per cent glucose and various concentrations of KH_2PO_4 were prepared. The initial pH values of the medium were adjusted to a uniform level prior to sterilization. The means of analyses of duplicate cultures (Table 3) showed that *C. funicola* rendered the

TABLE 3

EFFECT OF CONCENTRATION OF PHOSPHORUS AS KH_2PO_4 IN CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	MILLIGRAMS PHOSPHORUS IN CULTURE PER 35 CC. OF MEDIUM						
	00	1.81	7.02	21.13	42.0	104.53	211.3
	MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM						
0	92.7	92.7	91.6	92.0	92.3	92.2	90.1
6	91.2	89.3	80.8	82.6	83.8	87.2	87.0
13	85.4	73.1	73.8	70.4	73.2	76.6	70.4
22	89.1	64.7	60.3	63.9	62.2	59.6	49.4
	pH OF CULTURE MEDIUM						
0	4.4	4.4	4.4	4.6	4.7	4.9	4.9
6	6.4	7.4	6.4	6.0	5.6	5.5	5.3
13	6.8	8.0	7.8	6.7	6.4	5.8	5.6
22	6.2	7.4	7.5	6.9	6.5	6.0	5.8
	MYCELIUM FORMED, MILLIGRAMS						
6	0.65	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

medium progressively less acid with development under the different concentrations of KH_2PO_4 and that this change was more rapid with the lower concentrations of this salt. A greater increase in mycelial weight accompanied this greater rate of change of pH. Glucose dissimilation, however, did not follow this same trend but rather the inverse of it. More glucose was dissimilated per unit weight of mycelium at the higher than at the lower concentrations of phosphorus. Only slight development of *C. funicola* occurred in the complete absence of any phosphorus additions. The sustained growth obtained was attributed to the presence of phosphorus with the spores, although tests for the presence of inorganic ortho-phosphorus in the water spore suspension were negative. The optimum phosphorus concentration for *C. funicola* activity was approximately 7 mg. of phosphorus as KH_2PO_4 per 35 cc. medium, which represented the amount normally contained in Czapek-Dox medium. A reduction of phosphorus to 1.81 mg. per 35 cc. medium did not result in any decreased activity of the fungus.

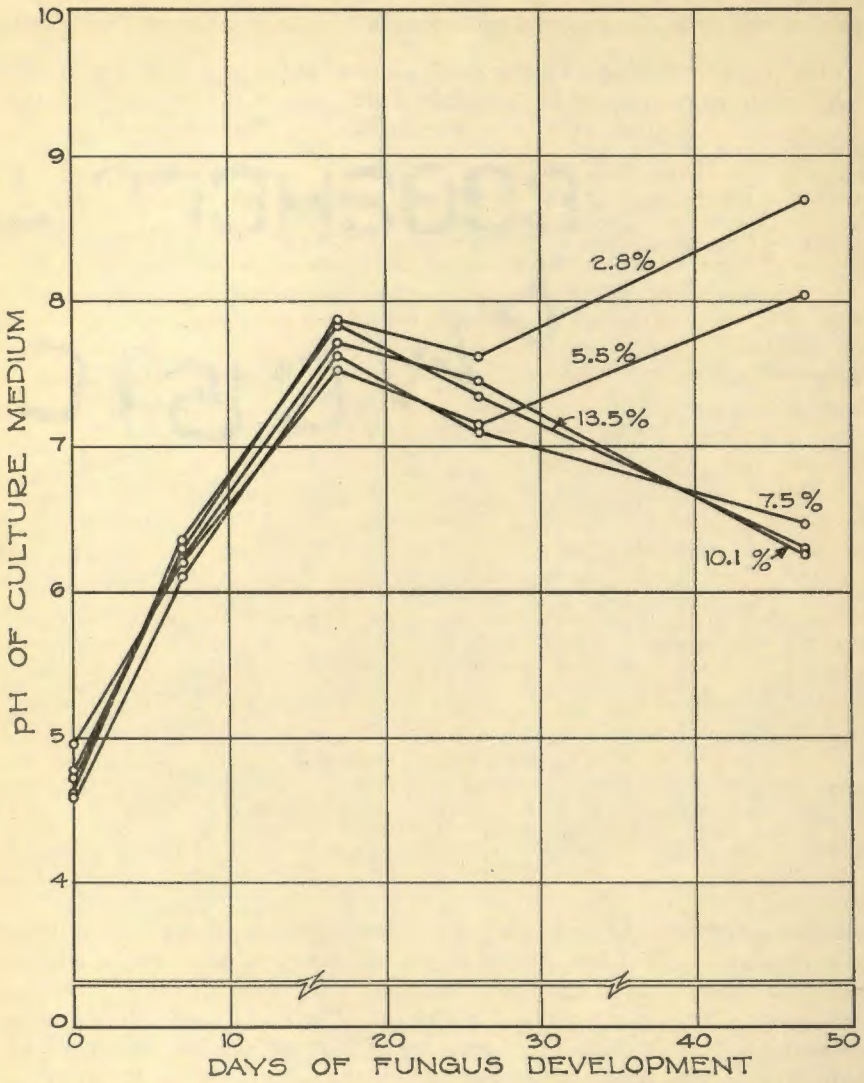

FIG. 3. Changes induced by *Chaetomium funicola* in pH of Czapek-Dox medium containing different initial concentrations of glucose.

VARYING THE INORGANIC NITROGEN SOURCE OF THE MEDIUM

The decrease in acidity of the medium in the foregoing experiments might be due either to the production of basic substances such as ammonia as suggested by Dickson (1) or merely to the utilization of nitrate ions from solution. In the absence of any initial formation of organic acids, the effect of the different inorganic nitrogen salts present in the medium would be expressed by a rise or fall in pH, depending on whether such

salts were nitrate or ammonium salts. To test this effect as well as to determine the relative availability of different inorganic nitrogen sources and their relation to the rate of glucose dissimilation, cultures were prepared containing 5 per cent glucose and the different nitrogen sources in amounts equivalent to 17.5 mg. elementary nitrogen (N) per 40 cc. of medium. The nitrogen solutions and the remaining portions of the medium were sterilized separately. Adjustments of the different nitrogen-containing media to a uniform initial pH were made in the non-nitrogen portions prior to sterilization. The mean results obtained by the analysis of duplicate cultures (Table 4) showed that with the exception of $NaNO_2$ all other tested inorganic nitrogen sources supported growth of *C. funicola*. The amount of glucose dissimilated paralleled the increase in mycelial weight of the fungus. Sodium nitrite apparently was toxic to *C. funicola* since no growth followed the addition of $NaNO_3$ after 3 months development. Ammonium salts supported growth only until the medium was rendered too acid to support further fungus growth. Thus, $(NH_4)_2SO_4$ and $(NH_4)H_2PO_4$ supported only slight growth because the medium reached a pH of about 3.7 and 3.5, respectively, very early in the development of the fungus, while $(NH_4)_2CO_3$ and $(NH_4)_2HPO_4$ supported greater growth because these salts, being basic, rendered the medium only very slowly acid. Nitrate salts such as $NaNO_3$ and KNO_3 proved most favorable for the development of the fungus because the change in pH shifted toward the alkaline side. Calcium nitrate yielded an unexpected result; only very slight growth of the fungus occurred on this salt during the first 28 days of development, after which a very rapid growth followed. The yield of mycelium obtained on this salt at the termination of the experiment was nearly as great as that obtained on $NaNO_3$ and KNO_3. On NH_4NO_3 even less mycelial yield was obtained than on the foregoing nitrate salts. With this salt the medium did not show any appreciable change in pH during the initial 16 days of fungus development but became more acid following this time, reaching pH 3.64 at the termination of the experiment. The greater availability of the ammonium radical over the nitrate radical followed from a consideration of the pH changes in the medium containing NH_4NO_3. The marked shift in pH to the more acid side in the later period of fungus development indicated the greater utilization of the ammonium ion, while the seemingly constant pH during the early period of development indicated the utilization of both ions to an equal extent. This latter conclusion may be modified by the tendency of the fungus to render the medium more alkaline, as occurred in the nitrogen-free cultures and those containing $(NH_4)_2CO_3$. A slight preferential utilization of the ammonium ion even in this early period was thus suggested.

SUMMARY

1. The effect of some modifications of Czapek-Dox medium on the rate of glucose dissimilation, mycelium formation, and pH changes in the medium by *Chaetomium funicola* Cke. was studied.

TABLE 4

EFFECT OF SOURCE OF INORGANIC NITROGEN IN CZAPEK-DOX MEDIUM ON GLUCOSE DISSIMILATION BY *Chaetomium funicola*

DAYS OF FUNGUS DEVELOPMENT	$(NH_4)_2CO_3 H_2O$	$(NH_4)_2SO_4$	$(NH_4)_2HPO_4$	$(NH_4)H_2PO_4$	NH_4NO_3	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	$NaNO_2$	NONE
				MILLIGRAMS GLUCOSE PER 1 CC. OF CULTURE MEDIUM						
0	51.5	51.5	51.4	52.5	52.5	50.5	52.7	51.5	.....	52.9
16	45.3	51.5	41.6	51.5	51.5	51.4	43.9	43.4	.....	52.6
35	33.6	51.6	37.0	48.9	36.0	41.2	22.8	19.8	.....	54.2
				pH OF CULTURE MEDIUM						
0	6.33	4.80	6.95	4.65	4.65	4.48	4.65	4.78	5.35	5.11
16	6.52	3.75	5.02	3.48	4.63	4.15	6.98	7.01	.....	5.69
35	3.95	3.67	3.67	3.47	3.64	6.38	7.23	7.23	.....	4.57
				MYCELIUM FORMED, MILLIGRAMS						
16	149.0	19.4	173.4	32.5	152.3	10.9	168.9	184.7	.....	13.6
35	273.4	20.0	213.6	43.0	217.8	325.6	403.5	445.2	.....	18.2

2. Changing the glucose concentration of the medium from 2.8 to 13.5 per cent revealed that glucose dissimilation followed a linear path, the rate of dissimilation being higher with the higher concentrations of glucose. The mycelial weights increased uniformly at all concentrations of glucose for approximately the first 17 days of development. Following this time at lower concentrations of glucose (2.8 per cent), the mycelial weights decreased, while at higher concentrations of glucose, the mycelial weights continued to increase. The pH of the media increased progressively to the alkaline side for 17 days of initial fungus development followed by a decrease. This decrease in pH was maintained for the duration of the experiment (47 days) in cultures containing 7.5 per cent and more glucose, while an increase followed in cultures containing less glucose but only after the glucose in the medium was reduced to a low level.

3. Changing the initial pH of the medium revealed that *C. funicola* grew readily over a wide range of pH with no growth at pH 2.12. Growth was reduced more at pH 2.90 than at 8.68. The pH changes in the medium together with the yields of mycelium suggested the optimum pH for development of *C. funicola* to be near neutrality and slightly on the alkaline side.

4. Increasing the concentration of KH_2PO_4 in the medium above the normal for the medium (35 mg. KH_2PO_4, or 7.98 mg. P per 35 cc. of medium) resulted in lower mycelial weights and slight increases in amount of glucose dissimilated. Decreasing the concentration of KH_2PO_4 to one-fourth of normal did not result in any appreciable decrease in mycelial weights or dissimilated glucose. The rate of change of pH of the medium was greater at the lower concentrations of KH_2PO_4 than at the higher.

5. Inorganic nitrogen salts markedly influenced the development of *C. funicola*. Salts such as $(NH_4)_2CO_3$, NH_4NO_3, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$, and $(NH_4)H_2PO_4$, which rendered the medium acid as the nitrogen ions became utilized, were definitely deleterious to the development of the fungus. The rapidity of action of this deleterious influence varied with the salt. Thus, $(NH_4)_2HPO_4$, $(NH_4)_2CO_3$, and NH_4NO_3 rendered the medium more slowly acid and hence were less deleterious to the development of *C. funicola* than were $(NH_4)_2SO_4$ and $(NH_4)H_2PO_4$. Sodium nitrate, KNO_3, and $Ca(NO_3)_2$, on the other hand, by rendering the medium alkaline, the condition favorable to the development of *C. funicola*, supported the greatest yields of mycelium. The late onset of fungus development on $Ca(NO_3)_2$ remains unexplained. The ammonium ion was utilized preferentially over the nitrate ion. Sodium nitrate did not support growth of the fungus and apparently was toxic.

6. The rate and amount of glucose dissimilation in general was closely associated with mycelium formation.

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CIRCULATION OF HEMOLYMPH IN THE WINGS OF THE COCKROACH, BLATTELLA GERMANICA L.[1]

III. Circulation in the Articular Membrane: The Significance of this Membrane, the Pteralia, and Wing Folds as Directive and Speed Controlling Mechanisms in Wing Circulation

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INTRODUCTION

In an earlier paper, the writers described circulation in normal wings of *Blattella germanica* (2); recently results of experimentally altering the normal circulatory pattern were reported (3). The above studies show that hemolymph in the German cockroach wing circulates in an orderly manner, with larger streams following definite hemolymph channels. Embryological and anatomical accounts of the origin and structure of the wing as a sac-like evagination of the body wall lead one to believe that hemolymph in the appendage is merely an overflow from the hemocoelic volume. However, observational studies (1, 2, 3) in the living insect show orderly currents in hemolymph movement and suggest a definite regulatory mechanism for directing the hemolymph over proper paths in the wing in order to maintain an uninterrupted flow. To prevent conflicting currents in a steady circuit of hemolymph movement throughout the appendage, presumption of such a mechanism is inevitable. Accumulated evidence (1, 2, 3) indicated that these regulatory properties lie in the wing base structures.

The present paper deals with circulation in the wing base (articular membrane); the base's influence on general currents in the channels of the wing proper; and certain circulation velocity controlling mechanisms. It is pertinent to remember that results herein reported are mainly observational, based on direction of hemolymph flow detected by light reflected from moving hemocytes, using the microscope as an aid. No histological section or micro-anatomical studies of directive structures were attempted. A few supplementary gross anatomical observations were made by dissection in connection with experimental sectioning. Detailed description of materials and methods has been given in previous reports (1, 2, 3). Terminology employed is essentially that used earlier and described elsewhere (1, 2). Additional terms are either shown in diagrams or defined in footnotes.

[1] Journal Paper No. J-919 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 649.

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RESULTS

Articular membrane[3] circulation is easily detected with a dissecting microscope, by using strong reflected light to discern direction of motion of hemocytes in the hemolymph stream. It is often difficult to observe movement through pigmented and sclerotized pteralia; however, observation of circulation in unpigmented intermediate areas, together with limited data concerning movement below the plates, has provided a better understanding of circulation in the articular membrane. In general, directions of circulation in the membrane of both sets of wings are similar.

1. Circulatory Patterns in, and Significance of, the Normal Articular Membrane

Articular Membrane of Tegmen (Fig. 1): Beneath the dorsal surface of the articular membrane, occupied by tegula, humeral plate, first, second, and part of the third axillary plates, and part of medial plates, there is a "mass" movement of hemolymph. This volume extends distad to the bases of longitudinal remigial veins where the mass movement becomes broken up into channel currents supplying the afferent remigial circulation.[4] The area below the pteralia is merely a sinusoidal extension of the hemocoele and has been called the anterior sinus. Hemocoelic hemolymph entering afferent remigial wing circulation gains access to the channels by diversion into the anterior sinus. The observed mass motion appears, therefore, to be a continuation of that in the hemocoele.

Hemocoele[5] circulation immediately below the membrane, between the axillaries and tergal plate margin is caudad (Fig. 1). As circulation proceeds back toward the posterior margin of the wing bearing segment, flow into the articular membrane (jugum) is prevented by fusion of dorsal and ventral membrane surfaces. On reaching the posterior margin of the tergite, the mass of hemocoelic flow is diverted mesad, and can be seen through the transparent intersegmental membrane immediately posterior to the tergal plate margin (Fig. 1).

The above mentioned fusion of surfaces involves that section of the articular membrane known as the jugum (free of axillaries) and a part

[3] Terminology: Articular membrane refers to the entire wing base membrane. The axillary membrane designates that part of the articular membrane containing pteralia (sclerites), while that part containing no sclerites is the jugum.

[4] Distinction between mass movement and channel movement is easily detected in behavior of hemocytes in the two areas. In mass flow, hemocytes move along easily and smoothly; in channels, cells jerk along as they contact the closely-confining channel limits. Differential velocity between hemocytes and hemolymph is greater in the channels than in the larger hemocoelic and anterior sinus spaces where obstruction to hemocytes is less.

[5] It is important to recognize that the depth of this circulation in the hemocoele could not be ascertained. It may be merely a thin sheet, primarily supplying the wing, and different essentially from underlying currents. That the "mass" is confined to the segment, as indicated by its mesad division near the posterior margin, suggests a possible hemocoelic arrangement of tissues aiding in the diversion of hemolymph into the anterior wing sinus. This point should receive further study to ascertain the exact sinus in which this sheet of flow occurs, and the depth to which it extends into the hemocoele.

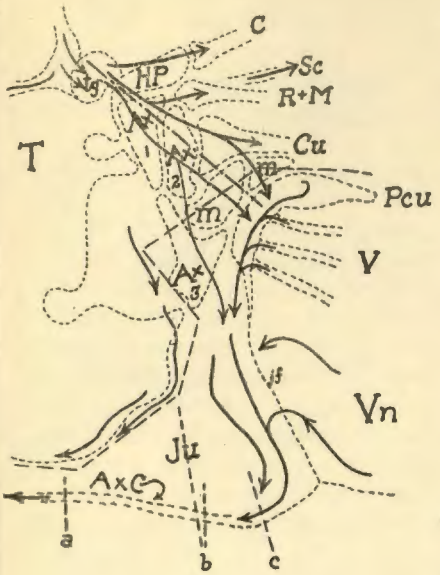

FIG. 1

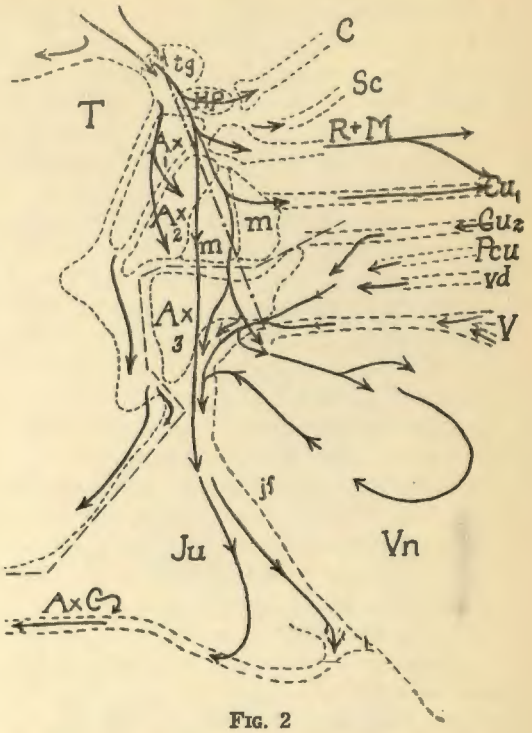

FIG. 2

FIG. 1. Diagrammatic representation of the anatomical arrangement and circulatory pattern in the articular membrane of the tegmen. Structures (pteralia, vein bases—channels, tergite, and jugal fold) are indicated by broken lines (short dashes); basal fold area by dot-dash line; area of fusion of articular membrane surfaces (dorsal and ventral) by broken (long dashes) zig-zag line [anterior sinus is cephalad to line; fused surface containing basal sinus (dilated channel) and jugal channels are caudad to line]. Circulatory pattern and directions of hemolymph movement are shown by arrows.

ABBREVIATIONS: a—indicates a section through the axillary cord (see text); Ax_1 to Ax_3—first to third axillary sclerites; Ax C—axillary cord; b—section through axillary cord and jugum (see text); c—section through jugal margin (see text); C—costa (abbreviations for all wing veins also indicate the corresponding channels); Cu—cubitus; HP—humeral plate; jf—jugal fold; Ju—jugum; m—medial plate; Pcu—postcubitus; R & M—common radial and medial base; Sc—subcosta; T—tergum or tergite; tg—tegula; V—vannal veins (and channels); Vn—vannal region; vd—vena dividens.

FIG. 2. Diagrammatic representation of the anatomical arrangement and circulatory pattern in the articular membrane of the hind-wing.

of the axillary section including Ax_1 and parts of both medial plates (Fig. 1). Unlike the anterior part (sinus), the fused jugal area contains a system of channels. At the base of the Pcu vein there is also a channel dilation, the basal sinus. Jugal circulation is entirely of efferent hemolymph returning to the general body circulation. The main source is vannal channel hemolymph. Often an additional source is hemocoelic flow passing over one or more short channels in the basal fold area (basal channels). This hemolymph usually enters the basal sinus, and either

joins efferent jugal currents (Fig. 1) or establishes secondary afferent flow in the anterior vannal channels, and returns by vannals near the posterior margin of the tegmen (1, 2, 3). The basal sinus has never been observed to empty into the Pcu channel in the normal tegmen, and it appears that such a connection is either inactive or does not exist. Its absence assists in the complete separation of afferent and efferent currents in the tegmen.

Jugal currents proceed posteriorly to the jugal margin (axillary cord) which they follow toward the general body circulation (Fig. 1). Immediately below the axillary cord hemolymph current within the hemocoele, flow is likewise in a mesad direction, but entirely independent of that coming from the jugal margin. Flow in the hemocoele is somewhat mass movement, while that in the cord is usually more like that in channel motion. Anatomically the axillary cord is a folded continuity of the intersegmental membrane. Observations on hemolymph flow show, however, that the cord channel is functionally discontinuous and completely separate from the hemocoele. It becomes looped (evaginated) and apparently fuses along a line with the segment so that its functional continuity with the hemocoele is lost. All available evidence indicates that it provides a closed vessel for transport of hemolymph from the jugum to the pulsatile organ region.

Articular Membrane of Hind-Wing (Fig. 2): The anterior sinus in the hind-wing is usually somewhat larger than that in the tegmen. In other respects axillary circulation in the two appendages is alike. Circulation in the jugal region is likewise similar; however, there is an essential difference involving the basal sinus. The hind-wing basal sinus is larger. As in the tegmen, it receives hemolymph from the hemocoele over basal channels, but unlike the tegmen it receives a large flow of efferent hemolymph from the remigium via Cu_2, Pcu, and vd channels. This route is an important outlet for hind-wing remigial streams. As in the tegmen, the remaining hind-wing remigial flow passes into the vannal area and flows efferently through vannal channels to the jugal region. All hemolymph in the remigium of the tegmen passes efferently through vannals (1, 2, 3) and omits entirely the basal sinus. Essentially as in the tegmen, hind-wing basal sinus hemolymph either passes into jugal currents, or establishes secondary afferent currents in certain vannals, and returns to jugal circulation via others.

2. Experimental Sectioning of Articular Membrane Channels

Sectioning regions of the articular membrane has aided in establishing its importance as a directive mechanism in wing circulation. Sectioning the axillary cord near the jugal fold will not arrest circulation (Fig. 1, with section indicated at "c"). Establishment of secondary flow in inactive channels carries the stream around or beyond the cuts. Cuts may be made even closer to the tergum without seriously impairing circulation (Fig. 1, indicated at "b" by short dash-line). When a section passes through the cord nearer the segment margin, establishment of secondary

currents is impossible; and, since all hemolymph must pass through the axillary vessel to complete the circuit (1, 2, 3), wing circulation is arrested (Fig. 1, with a cut at "a"). The axillary vessel separation is the most positive and definite way of permanently arresting wing circulation.

Other sections can be performed to alter circulatory patterns, but few arrest circulation. A proper cut extending from the axillary vessel toward the anterior sinus usually stops flow (Fig. 1, indicated at "b" by long broken line).

3. Significance of Pteralia and Wing Folds

Pteralia: The pteralia give support to the articular membrane, and facilitate entrance of hemocoelic hemolymph into the channels by keeping the membranous surfaces separated. Their function is especially important in the folded wing. Experimental pressure at these sclerotized areas (simulating fused membrane surfaces) retards or arrests circulation. Circulation is likewise affected when a wing is changed from flight position to that of rest (folded), which indicates that the presence of these structures may serve to alter the velocity and volume of flow through the wing. The sclerites also prevent collapse in the basal sinus area, where slight pressure retards or impairs circulation.

Wing Folds: Manipulation of the various wing folds (basal, vannal, and jugal folds) indicates that they have a decided influence on the speed of circulation. When a wing is outstretched in a position simulating flight,[6] the folds are "opened" and circulation is accelerated; on folding to a normal resting position, streaming is arrested. Wing creases offer considerable resistance to flow because of stress put on folded or "doubled back" channels. During this strain on the channels the pteralia assist in preventing complete closure of channels and sinuses in the wing base.

The vannal fold in the tegmen is "vestigial" and plays little part in circulation; however, it extends entirely across the hind-wing. Pressing against a hind-wing along the fold retards vannal currents. Remigial flow above may continue because of efferent flow in the Cu_2, Pcu, and vd. The influence of the jugal fold on the volume and velocity of circulation is the least difficult to observe. Several channels cross the membrane at this point. When these channels are creased (resting position) or when undue folding pressure is exerted, circulation in the immediate area is arrested. By proper manipulation of this fold it is sometimes possible to arrest circulation in the tegmen. Often stresses on one fold accelerate streaming in other areas, so long as there is an efferent outlet for hemolymph.

Invariably, struggling of specimens attempting to free themselves markedly influences the rate of flow. Sometimes the flow is accelerated; at other times it is retarded, or even arrested. The active muscular

[6] Care must be exerted in outstretching the wing to a natural flight position. Forcible outstretching often retards circulation because of unnatural strains on the channels and other parts.

movements along with the compression and enlargement of the roach's body in such struggling undoubtedly alter the hemocoelic pressure and thus affect circulation in the appendages.

In view of the observation that a wing carefully outstretched to simulate flight position, with all folds and undue stresses "eliminated," possesses an accelerated flow, it is not unlikely that wing circulation may be at a much higher rate during flight. Evidence collected through many observations indicates that speed of hemolymph flow during flight is influenced by the presence of the basal mechanisms and wing folds.

4. Significance of Reversed Circulation

Reversed circulation has a bearing on the definiteness of the hemolymph circuit and the accompanying potential necessary for its movement. Apparently, it sometimes happens that reduced pressure in the hemocoele allows afferent remigial hemolymph to "rush" or flow back into the hemocoele. A reduction in hemocoelic pressure may result from sudden expansion of the body. Generally, reverse in remigial flow is not accompanied by a corresponding reverse in vannal circuit (1, 2). Failure of the vannal efferent currents to reverse may indicate a probable "aspiratory" action of the pulsatile organ in maintaining a steady reduction gradient from anterior sinus to pulsatile organ (1, 2). Normally heart action seems to produce a positive pressure acting synchronously with the negative pressure of the pulsatile organ. The observational evidence suggests that proper distribution of these pressures in maintaining steady and uninterrupted hemolymph flow lies with the articular mechanism.

DISCUSSION AND SUMMARY

Study of normal wing circulation (1, 2) has shown that outgoing (afferent) hemolymph flows in channels along the anterior wing margin to the distal apical margin. Afferent flow changes its course in certain definitely outlined wing areas (intermediate circulatory zones), and is forced proximally (efferently) in channels along the posterior wing margin (1, 2). This general and regular flow suggests that a definite circulatory potential is established, whereby the action of hemolymph propelling forces is utilized to maintain a pressure gradient. Preceding successful establishment of this gradient it is necessary that a definite anatomical arrangement of structures be present at the wing base to direct hemolymph through definite channels, and to prevent obstruction of flow by random mingling of opposing currents. The arrangement of the anterior sinus and fusion of the articular membrane about efferent channels evidently provides this type of directive mechanism.

Should the wing be an unmodified sac-like evagination of the body wall, void of hemolymph directing mechanisms, it is not likely that there would be a steady and uninterrupted flow of hemolymph throughout channels of the appendage proper. Equal pressure at all points of a wing base not possessing a directive mechanism would merely result in an inflated, hemolymph-filled appendage, lacking a high and low pressure

area capable of influencing direction and movement. This simple condition can be simulated in the normal wing by sectioning certain vital areas, whereby the potential influence of the propelling organs (3) is destroyed. Failure to observe *complete* reversal in either normal wing circulation, or under experimental conditions, lends added support to the importance of the axillary structure in directing hemolymph and in distributing properly the force propelling it (1, 2, 3).

CONCLUSIONS

1. Two "types" of circulatory movement are found in the articular membrane region; namely, mass and channel movement. The former is confined to larger spaces, such as the adjoining parts of the hemocoele, and the anterior sinus; the latter movement is confined to channels. Behavior of moving hemocytes within channels is different from that of those circulating in the spacious hemocoele. This observation is of considerable assistance in ascertaining the influence of the articular membrane and its contained structures on circulatory direction.

2. Hemocoelic hemolymph enters afferent wing channels through the anterior sinus, a "dilation" of the axillary membrane.

3. Fusion of the posterior area of the articular membrane (jugum) prevents intermingling of hemocoelic and anterior sinus hemolymph with returning efferent wing currents. The fused membrane contains "closed" channels which provide an outlet for efferent hemolymph.

4. Efferent hemolymph flows from the jugum along the axillary cord channel into the pulsatile organ region before returning to the general circulation.

5. Anatomical features of the articular membrane provide an effective mechanism which takes part in allowing an uninterrupted stream of hemolymph to flow through the wing. The mechanism thus acts as an accessory in maintaining a potential or circulatory gradient. Such a gradient is a physical prerequisite for the steady transport of hemolymph through the wing.

6. By cutting through certain vital points along the axillary cord, circulation can be arrested. This operation amounts to the destruction of the circulation potential by the removal of a return pathway. The wing then becomes a sac-like evagination of the body wall, void of a circulatory potential or gradient.

7. The pteralia located in the articular membrane assist in keeping open the circulatory passages through which hemolymph enters the wing.

8. Wing folds have a regulative influence on the velocity of circulation in that, when the folds are closed, much less hemolymph is able to pass across the crease.

9. Evidence indicates that circulation is much more rapid during flight than when at rest. When in a resting position the wings are folded and the creases "obstruct" or retard circulation. When folds are "eliminated" by holding the wing in a simulated flight position, circulation is accelerated.

10. There is only one important difference between the fore- and hind-wing bases. The difference involves the hind-wing basal sinus which, unlike that of the tegmen, receives remigial hemolymph.

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THE CHILEAN RHOPALIDAE IN THE EDWYN C. REED COLLECTION (HEMIPTERA)

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Through the courtesy of Professor Carl J. Drake it has been my privilege to work over the Rhopalidae represented in the Edwyn C. Reed collection, now a part of the Drake hemiptera collection. The study of this material, which formed the basis for Reed's treatment of the group in his "Sinopsis de los Hemipteros de Chile" has contributed much to my understanding of the forms described by Spinola and Blanchard in Gay's Historia de Chile as well as those Chilean forms discussed in the writings of Dallas and Signoret. As indicated below the three names erected by Reed, namely *Harmostes signoreti* Reed, *Harmostes gracilis* Reed, and *Corizus chilensis* Reed, must fall as synonyms of earlier described species.

Harmostes raphimerus (Spinola)

1852 *Merocoris raphimerus* Spinola, in Gay, Hist. de Chile, Zool. VII: 164.
1863 *Harmostes raphimerus* Signoret, Ann. Soc. Ent. Fr., (4)3:560.
1900 *Harmostes raphimerus* Reed, Sinop. Hemip. de Chile, p. 51.
1900 *Harmostes signoreti* Reed, Sinop. Hemip. Chile, p. 52.
1917 *Harmostes raphimerus* Gibson, Ent. News, 28:446.
1941 *Harmostes raphimerus* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36:84.

Head longer than wide across eyes (37 : 30), the tylus thin and high. Antenniferous tubercule spines slender, acute, diverging anteriorly, the distance from front of eye to apex of spine noticeably greater than length of an eye. Antennae faintly shorter than head, pronotum and scutellum conjoined; segment I from above surpassing tylus by half its own length; II enlarged and somewhat compressed on basal part; proportions, 17 : 25 : 32 : 19 (in some, 17 : 30 : 40 : 20). Bucculae sloping backwards, disappearing in front of a line through front margin of eyes. Rostrum reaching on metasternum, segment I not going beyond hind margin of eyes. Pronotum strongly widened posteriorly, the sides concavely sinuate, the edge sharply reflexed so that there is formed a broad channel within the lateral margin (in some specimens the edge is not reflexed toward the humeri and the lateral channels, therefore, are prominent only along the front half), disc with a distinct median carina, front angles conspicuously produced, humeral angles broad. Scutellum faintly broader than long. Hemelytra with entire clavus and corium except for small elongate area in middle cells of the latter, coriaceous and coarsely punctate; membrane hyaline, sometimes speckled with brown.

LENGTH, 9.8–10.1 mm. WIDTH (across humeri), 3.4 mm.

Several examples of this species are present in the Reed collection and are labeled "*Harmostes raphimerus.*" One specimen bears the nota-

tion "Sp. nov. or near *raphimerus,* 1 ant ½ head." Other specimens, apparently specifically inseparable from those mentioned above, are labelled *signoreti.* One of these, a female, carries the pin label *"Margus sp. nov."* and is presumed to be the type. I have designated this specimen *lectotype.*

It seems evident that Reed based his *signoreti* more on Signoret's description and the inconsistencies between it and Spinola's description than upon any characters he himself was able to discern in the specimens before him. Reed correctly pointed out that Spinola's type specimen must have been a female even though it was described as a male. Spinola's characterization "antennarum articulo primo plus capite longiore" of course is not literally true and puzzled Reed much, and this one character perhaps more than any other led him to create the name *H. signoreti.* I think Spinola really meant that the first antennal segment projected much beyond apex of head. Signoret must have recognized this for he says, "Antennes avec le premier article tres petit, a peine le tiers de la tete." Although the segment is short and stout and appears to be no more than a third as long as the head, when it is subjected to actual measurement under present day techniques it proves to be decidedly more than a third as long as the head. Antennal II is short, distinctly swollen and somewhat compressed at the base; in some examples it is not as long as distance from apex of tylus to a line across base of eyes, whereas in others it is about as long as from apex of tylus to the ocelli.

Gibson has placed *Harmostes montivagus* Distant as a synonym of *raphimerus* Spinola, but size alone would seem to preclude this. Torre-Bueno has followed Reed in considering the discrepancies between Spinola's and Signoret's descriptions and considers that two species are involved. However, it is presumed that Signoret had Spinola's types available for study. It is true also that there is some variation in the antennal segments, both in relative lengths of the segments and in the degree of enlargement of the base of the second segment, and that the flattened nature of this segment is not evident when the antennae are viewed from certain angles. In view of all these things, I am forced to consider *signoreti* Reed an outright synonym of *raphimerus* (Spinola).

Harmostes minor (Spinola)

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1917 *Harmostes minor* Gibson, Ent. News, 28:448.
1941 *Harmostes chilensis* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36:85.

Head subequally as long as broad across eyes (25 : 24). Antennae short, not as long as head, pronotum and scutellum conjoined; segment I as seen from above projecting beyond tylus by half its length; proportions, 17 : 19 : 20 : 15. Spines of antenniferous tubercules fairly prominent; from above slender, directed straight forward. Bucculae tapering

posteriorly, extending a little beyond a line through front margin of eyes. Rostrum reaching to a point between middle coxae; basal joint not quite attaining base of head. Pronotum trapezoidal, the sides almost straight, somewhat expanded and reflexed so that there is a broad sulcus within for the full length, the edge itself minutely granulate; front angles slightly produced, humeral angles rather broad; distance across humeri not greater than distance across base of hemelytra. Scutellum subequally as long as broad, its margins raised, the apex broadly rounded. Clavus and exocorium opaque and coarsely punctate, mesocorium hyaline, impunctate except for a marginal row. Membrane hyaline, without specks or dark streaks. Apex of last genital segment of male broadly excised beneath; the clasper small, its tip dark and recurved.

LENGTH, 6.1–7.4 mm. WIDTH (across humeri) 2.0–2.5 mm.

There are several specimens in the collection, and one bears the label "*Harmostes minor?*" These examples agree well with Dallas' description of *chilensis* and also fairly well with the description of *minor* except for size, and, of course, the hind femora are armed. Just what Spinola meant when he said "pedibus inermibus" is quite puzzling, and if he were correct in so characterizing his specimens the species must be referred to some other genus. It is presumed, however, that Signoret saw Spinola's types, and he referred them to *Harmostes* and placed *chilensis* Dallas as a synonym. I have found no evidence to support Reed's statement that Gay's Volume 7 did not appear until 1853 or 1854 and have, therefore, followed Signoret and Stal in giving precedence to Spinola's name.

The species is related to *Harmostes procerus* Berg.

Harmostes marmoratus Blanchard

1852 *Merocoris marmoratus* Blanchard, in Gay, Hist. de Chile, Zool. 7:166.
1863 *Harmostes marmoratus* Signoret, Ann. Ent. Soc. Fr., (4) 3:561.
1900 *Harmostes marmoratus* Reed, Sinop. Hemip. Chile, p. 53.
1900 *Harmostes marmoratus* Berg, Anal. Mus. Nac. Buenos Aires, 7:85.
1917 *Harmostes marmoratus* Gibson, Ent. News, 28:446.
1941 *Harmostes marmoratus* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36:86.

Small, slender; greenish yellow, usually with a series of brownish spots along expanded margin of pronotum and hemelytra, sometimes strongly roseate or the pronotum and clavus in part strongly embrowned. Head subequally as broad as long (22 : 23), the anteocular part to apex of antenniferous tubercule as seen from the side barely as long as an eye. Antennae subequally as long as head, pronotum and scutellum combined; segment I surpassing apex of tylus by less than half its own length; proportions, 12 : 17 : 19 : 15. Antenniferous tubercules from above slender, not noticeably divaricating, from the side rather stout and rectangular with dorsal edge slightly produced. Rostrum not attaining hind coxae, segment I barely reaching beyond a point opposite middle of eye. Bucculae sloping posteriorly, terminating opposite front of eye. Pronotum distinctly raised and widened behind front lobe, the median length just one-half width across humeri, the lateral margins narrowly

expanded and reflexed; the anterior angles short, acute; the humeral angles rounded, the distance across them not greater than width across base of hemelytra. Scutellum as long as broad, its margins raised. Hemelytra with entire clavus and corium, except for distal half of middle basal cell, strongly punctate. Membrane hyaline, immaculate. Genital capsule of male with its posterior margin roundly convexly produced at the middle between the projecting claspers. Claspers recurved apically as in *H. minor,* but smaller and with different apex.

LENGTH, 5.5–6.1 mm. WIDTH (across humeri), 1.6–2.0 mm.

This form is closely related to *H. minor.* It is, however, smaller and slenderer, the lateral pronotal margins are less broadly expanded and more sharply reflexed, the pronotum is more suddenly widened so that its lateral edge is distinctly concave (especially noticeable in the male), the tylus extends beyond middle of first antennal segment, the inner and distal cells of corium are punctate, and the male genital characters are distinctive. As in *rhaphimerus* and *minor* there is much variation in color, the pale, more or less immaculate examples being easily confused with *H. minor.* Judging by the specimens, I have seen Gibson was in error in saying the membrane is spotted with fuscous. Torre-Bueno has followed Gibson in this even though there is no mention of such in the descriptions of Blanchard, Signoret, and Reed. *Harmostes corazonus* Distant, which Gibson placed as a synonym of *marmoratus,* is described as having a speckled membrane and for the time being must be considered as a valid species.

Xenogenus gracilis (Reed)

1900 *Harmostes gracilis* Reed, Sinop. Hemip. Chile, p. 53.
1941 *Harmostes gracilis* Torre-Bueno, Bull. Brooklyn Ent. Soc., 36:84.

In the Reed collection under the name *Harmostes gracilis* there are several examples of a species that belong to *Xenogenus* Berg. That Reed was in doubt about the generic position of the species is evident by the fact that one of his specimens carries the label "nov. gen. et sp. near Harmostes." This specimen is a male in good condition and I have designated it *lectotype.*

Xenogenus gracilis (Reed) is close to *X. picturatum* Berg and *X. extensum* Distant, and a study of a long series of examples from many localities will be necessary for an understanding of their true relationships. In Reed's Chilean examples the male clasper is swollen at the middle and constricted before the apex as in examples of *X. extensum* from Arizona but is decidedly larger.

Liorhyssus hyalinus (Fabr.)

1794 *Lygaeus hyalinus* Fabricius, Ent. Syst., 4:168.
1835 *Corizus gracilis* Herrich-Schaeffer, in Panzer, Fauna Germanica, p. 127.
1842 *Corizus truncatus* Rambaur, Fauna Andal., 2:144.
1852 *Merocoris lineatoventris* Spinola, in Gay, Hist. de Chile 7:168.
1852 *Merocoris maculiventris* Spinola, Gay, Hist. Chile, 7:170.
1852 *Merocoris microtomus* Spinola, Gay, Hist. Chile, 7:171.
1852 *Merocoris rubescens* Blanchard, in Gay, Hist. de Chile, 7:173.

1859 *Corizus gracilis* Signoret, Ann. Soc. Ent. Fr., (3) 7:88.
1859 *Corizus quadrilineatus* Signoret, Ann. Soc. Ent. Fr., 7:90.
1870 *Liorhyssus hyalinus* Stal, Enum. Hemip., 1:222.
1900 *Corizus gracilis* Reed, Sinop. Hemip. Chile, pp. 54, 55.
1900 *Corizus chilensis* Reed, Sinop. Hemip. Chile, p. 55.
1900 *Corizus maculiventris* Reed, Sinop. Hemip. Chile, p. 55.
1900 *Corizus microtomus* Reed, Sinop. Hemip. Chile, p. 56.
1900 *Corizus lineatoventris* Reed, Sinop. Hemip. Chile, p. 56.
1900 *Corizus quadrilineatus* Reed, Sinop. Hemip. Chile, p. 57.
1900 *Corizus gracilis* Berg, Anal. Mus. Nac. B. Aires, VII: 85.

In the Reed collection there are a number of specimens of *Liorhyssus hyalinus* (Fabricius) representing many of its color variations. These were segregated by Reed and stand under the name labels attached by him as indicated below.

Corizus gracilis is so labeled. In the literature this name has already been synonymized with *hyalinus* Fabricius.

Corizus chilensis is represented by three specimens each bearing the label "*rubescens.*" One specimen is labeled "Salta, 19-11-87, *rubescens* = *chilensis.*" These agree fairly well with Reed's and Blanchard's characterizations and seem to be specifically inseparable from *hyalinus.* The names *Merocoris rubescens* Blanchard and *Corizus chilensis* Reed must therefore be considered as synonyms of *hyalinus* Fabr.

Corizus maculiventris (Spinola) is represented by four specimens which are specifically identical with those labeled *rubescens* though they are not so strongly marked above with red, and the ventral black patch is larger, extending well back on the venter. In general these Chilean specimens are a little larger and stouter than specimens of *L. hyalinus* from North America and Europe. The name *maculiventris* may well be retained as a varietal name when the color forms of this almost cosmopolitan species are worked out.

Merocoris microtomus Spinola is only a pale form of *L. hyalinus.* The specimens in the Reed collection agree well with the descriptions of both Spinola and Reed.

Merocoris lineatoventris Spinola and *Corizus quadrilineatus* Signoret constitute a color variety of *hyalinus* in which the venter is marked with four more or less distinct longitudinal dark stripes.

Anhyssus tricostatus (Spinola)

1852 *Merocoris tricostatus* Spinola, in Gay, Hist. de Chile, 7:172.
1859 *Corizus annulatus* Signoret, Ann. Soc. Ent. Fr., (3) 7:98.
1859 *Corizus tricostatus* Signoret, Ann. Soc. Ent. Fr., (3) 7:104.
1900 *Corizus tricostatus* Reed, Sinop. Hemip. Chile, p. 57.
1900 *Corizus annulatus* Reed, Sinop. Hemip. Chile, pp. 54, 57.

In the Reed collection there are specimens of this form labeled "*annulatus.*" Reed himself, however, considered Signoret's species as probably identical with Spinola's, an opinion in which I concur. The species is recognized by its small size, the long, rather upright clothing hairs, the speckled legs, antennae and wing veins, the maculate connexivum, the short antennae, and the characteristic claspers of the male. The apical antennal segment is pale at base and apex.

Niesthrea fenestratus Signoret

1859 *Corizus fenestratus* Signoret, Ann. Soc. Ent. Fr., (3) 7:93.
1900 *Corizus fenestratus* Reed, Sinop. Hemip. Chile, p. 56.

There are no specimens in the Reed collection that can be placed as *fenestratus*. A careful study of the description leaves me with the impression that the species probably should be referred to *Niesthrea*.

FLUORESCENT BACTERIA IN DAIRY PRODUCTS[1]

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Fluorescent bacterial colonies occasionally are noted on agar plates prepared from dairy products, particularly when certain media are used and the plates are incubated at temperatures below 37°C. Addition of the responsible organisms to milk, cream, and certain derivatives of them commonly results in objectionable but variable changes.

Direct plating can detect fluorescent bacteria only when they are so abundant in a product that they are not diluted out in plates showing a good colony distribution; accordingly, it gives an inadequate idea of the distribution of the organisms. The work herein reported deals (a) with the distribution of the fluorescent bacteria in dairy products as shown by enrichment procedures and (b) with the action of the organisms on milk and butter.

HISTORICAL

Various investigators have pointed out the conspicuous action on milk and butter of fluorescent bacteria isolated from different materials.

From a sample of cheesy butter Krueger (7) obtained various organisms, one of which was stated to be *Bacillus fluorescens non-liquefaciens,* although it was described as a non-motile rod that produced terminal spores and grew both aerobically and anaerobically. At 16° to 18°C., the optimum temperature, a foul fermentation was quickly produced in sterile milk; the liquid developed a yellow color with a green fluorescence and finally became slimy; the reaction gradually turned acid and after 10 days there was a penetrating odor of trimethylamine; ammonia and hydrogen sulfide also were formed. Butyric and formic acids were produced from triglycerides; it appeared that the higher molecular weight fatty acids were decomposed to these two acids during fat hydrolysis.

Lafar (9) isolated an organism that produced rancidity in butter when inoculated into the cream before churning and named it *Bacillus butyri fluorescens;* however, Reinmann (18) found this organism to be *Bacillus fluorescens liquefaciens.*

Reinmann (18) inoculated *Bacillus fluorescens liquefaciens* into butter churned from sterile cream. The butter had a fresh, desirable flavor when first prepared but developed a strong undesirable odor in a few days and was inedible in 2 or 3 weeks, although not rancid.

Schreiber (20) noted that *Bacillus fluorescens liquefaciens* was the organism most frequently encountered on agar plates prepared from sur-

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faces of cylinders of butter that had been buried in the soil for various periods. Five cultures of the organism, isolated from soil and water, all decomposed cylinders of sterile butter at room temperature when inoculated on the surfaces. Cultures that were lipolytic when first isolated lost their ability to attack fat after being carried on gelatin for 1 year and 9 months. Schreiber stated that in the presence of nutritive material and oxygen the organism breaks down fat and destroys the free fatty acids after they react with calcium carbonate.

Laxa (10) found that when *Bacillus fluorescens liquefaciens* had grown in butter for 1 month the total acid number of the butterfat was 197.4 and the volatile fatty acid number was 0.94; the control fat had a total acid number of 12.2 and a volatile fatty acid number of 5.05. The data were considered to indicate that the organism splits primarily glycerides of the non-volatile fatty acids. Laxa believed that the organism was unable to decompose the higher fatty acids to butyric and formic acids, as suggested by Krueger (7).

Several microorganisms were investigated by Orla-Jensen (16) from the standpoint of their importance in the development of rancidity in butter under commercial and laboratory conditions. *Bacillus fluorescens liquefaciens* was always found in fresh butter, while *Bacillus fluorescens non-liquefaciens* was encountered only occasionally; rancid butter often contained the former organism but not the latter. *Bacillus fluorescens liquefaciens* was one of the two predominating liquefying bacteria in the surface layers of sweet cream butter held at room temperature for 3 days but was present only in small numbers in sour cream butter similarly held. It did not grow in the interior of butter and died off in the surface layers when the volatile fatty acid number reached about 3.5. Butter made from sterile sweet cream had an objectionable taste and a butyric acid-like odor after 1 week and was completely inedible after 2 months. The organism supposedly decomposed butterfat uniformly but utilized the non-volatile fatty acids in preference to the volatile ones. Milk-souring bacteria did not prevent the growth of *Bacillus fluorescens liquefaciens* in butter but, when sufficient acid had developed, hydrolysis of the fat was believed to be retarded. The addition of 2.9 per cent salt (21.6% brine) to butter prevented growth and fat hydrolysis by the organism. Since *Bacillus fluorescens liquefaciens* is widespread in water, it was believed to be introduced into butter from this source. Pasteurization of cream at 85°C. destroyed all organisms injurious to butter.

Kruyff (8) isolated nine species of fat-splitting bacteria from soil, sewage, water, old butter, and animal feces, all of which grew at 37°C. The only one studied to any extent was identified as *Bacillus fluorescens liquefaciens.*

Wolff (25) obtained *Bacterium fluorescens* from part of the milk samples examined but only after they had been held at a low temperature (5° to 7°C.) for several days (2 to 7). Under these conditions fluorescent bacteria comprised from 22 to 42 per cent of the total flora of certain samples. When the samples were held at 20°C., fluorescent colonies were encountered only occasionally on plates poured with the milk.

According to Barthel (1), *Pseudomonas fluorescens* splits fat to cause rancidity but is incapable of attacking glycerol.

Luxwolda (14) grew *Streptococcus lactis* and *Bacterium fluorescens liquefaciens* together in milk and found that at 10°, 13°, or 15°C. both species appeared to profit by the association. After 6 days at 15°C., the milk was sour and coagulated and contained 1,700,000,000 *Streptococcus lactis* and 4,000,000 *Bacterium fluorescens liquefaciens* organisms per ml. Since *Bacterium fluorescens liquefaciens* lived in a sour medium, it appeared that the milk-souring bacteria produce something besides acid that hinders the growth of the fluorescent organisms in sour milk. *Bacterium fluorescens liquefaciens* produced a rennin coagulation and then peptonized the milk. At 3° to 5°C. the odor and taste of milk inoculated with the organism remained completely normal up to 19 days, even though it contained over 300,000,000 bacteria per ml., but after 20 days the milk was bitter and coagulated with alcohol.

Gubitz (4) isolated several cultures of *Bacterium fluorescens* and related organisms, which included *Bacterium punctatum* and *Bacterium putidum*, from milk, butter, soil, water (from various sources), and plants and studied their growth temperatures. The optimum temperature of the organisms was under 30°C., and they were found to be one of the important types that grow at 0°C. Cultures of *Bacterium fluorescens* with a maximum growth temperature of about 35°C. were designated warm forms, while those with a maximum growth temperature of about 27° to 30°C. were termed cold forms. The maximum and optimum temperatures of these organisms were reduced about 5°C. from the original by growth in liquid medium for several generations at 0°C. The physiological properties, particularly the lipolytic and proteolytic characteristics, were not altered by holding at 0°C. for considerable periods. Growing the warm forms for 24 transfers at 30°C. raised the optimum and maximum growth temperatures about 5°C. Two types of colonies—(a) bluish, lobated, irregular-shaped, and spreading and (b) round, raised, discoid, smooth, and glassy-appearing—were produced by the fluorescent organisms. *Bacterium fluorescens* developed an intense bitter taste in milk in 2 days at 18°C. and later peptonized the milk; hydrolyzed fat at 12° and 18°C.; failed to produce hydrogen sulfide; gave only weak growth in media containing 5 per cent sodium chloride; grew at a pH as low as 5.4 to 5.8; and was destroyed at 63° to 64°C. for 30 minutes.

Shutt (21) found that contaminated water used in washing butter was responsible for an unclean, putrefactive flavor which developed on the surface of the butter during holding. The off flavor appeared chiefly during the spring and summer months and was particularly common after periods of heavy rains. It was noted in butter from creameries having good water supplies. In creameries having difficulty with off-flavored butter the water supplies contained large numbers of putrefactive bacteria, chief of which was *Pseudomonas fluorescens*. Sterile butter inoculated with this organism developed the typical surface flavor in 28 days at 25°C. Heating the water to 87.8°C. for 10 minutes was necessary to destroy the organism. The trouble disappeared when the water was

treated or when pure water was substituted for a contaminated supply. Neutralizing the cream to not less than 0.35 per cent acid was beneficial in avoiding the defect, since the organism grew but feebly at pH 6.6. Surface taint occurred only in sweet cream or neutralized cream butter and never developed in sour cream butter.

Löhnis (11) reported that *Bacterium fluorescens* and closely related forms, which regularly are present in water, play an important role in the development of rancidity in butter stored with access to air. Since the organisms grow at low temperatures and decompose fat and protein, they also were detrimental to the flavor of milk.

Newman (15) examined three samples of milk with a bitter flavor and found that they contained mainly Pseudomonas organisms. The bacteria grew well at 4°C. and produced a fluorescent blue-green, yellow, or red pigment on agar plates. Colonies picked into sterile milk produced a strong quinine-like bitterness in the milk in 24 to 48 hours at room temperature. A different species of Pseudomonas was isolated from each sample of milk. One of the organisms corresponded to *Pseudomonas ovalis.*

Orla-Jensen (17) reported that *Bacterium fluorescens liquefaciens* frequently played an important part in the development of rancidity in butter. *Bacterium pyocyaneum,* however, grew so slowly at ordinary temperatures that it did not spoil butter under normal conditions. Since *Bacterium fluorescens liquefaciens* often is added to butter by wash water or ice, Orla-Jensen advised pasteurizing wash water or treating it with chlorine. He stated that this organism produces a turnip, tallow, and sometimes a soap flavor in milk; turnip flavor frequently was noted in milk that had been held at a low temperature.

Rumment (19) stated that numerous investigators have demonstrated *Bacterium fluorescens liquefaciens* was a usual inhabitant of polluted water and caused rancidity in butter. The organism was used in experimental work to determine the number of microorganisms that passed from the wash water into the butter and also the effect of these organisms on the keeping quality of butter. Sweet cream butter absorbed more organisms from the wash water than sour cream butter; the firmer the consistency and the larger the butter granules the fewer were the bacteria traceable to the wash water; and in sweet cream butter (pH 6.8) the organism increased rapidly and decomposed the fat intensely at higher temperatures but only slightly at lower temperatures, while in sour cream butter (pH 4.2 to 4.3) it did not increase, and the fat remained unchanged in the cold but developed an unclean, tallow flavor at higher temperatures. The fatty acids formed in butter were found to have a germicidal effect on the organism.

Virtanen (24) listed the more common defects produced in butter by bacteria as fermented, cheesy, putrid, and "rank." The enzymes causing the defects were stated to be formed by proteolytic water bacteria of the *Pseudomonas fluorescens* and *Pseudomonas punctatum* groups. These bacteria were not easily destroyed by heat but were inhibited by

the acidity of sour cream butter and by salt. It was noted that they usually do not cause defects when the water supply is uncontaminated and the milk is delivered daily; when the milk or cream is 2 or 3 days old, difficulty may arise even though the water supply is pure. The enzymes of these bacteria were not destroyed during pasteurization of the cream and sometimes caused defects in butter in the absence of living bacteria. The catalase test for butter was recommended as a test for the presence of proteolytic bacteria, although a negative test was no assurance that spoilage would not occur.

Henneberg (6) reported that protein decomposition without acid production is typical of the fluorescent group of bacteria, even though many cultures can ferment lactose and dextrose. Protein decomposition, however, can be largely inhibited by the presence of sugar, as is illustrated by the fact that gelatin-liquefying cultures sometimes do not liquefy gelatin when sugar is added. Air favors an alkaline development, while lack of air favors an acid fermentation. Of two gram-negative, gelatin-liquefying, fluorescent organisms, one produced a bitter putrefaction in milk and the other a soap flavor; both strongly hydrolyzed fat; both produced a putrid odor and ammonia in peptone broth; both grew well at 6° to 8°C.; and 7.5 per cent salt was endured by one organism but only 5 per cent by the other. It was stated that *Bacterium fluorescens* and other alkali-forming bacteria are important in decomposing the fat and protein of butter, but low temperatures, acid, and salt are preserving factors.

The ability of *Pseudomonas fluorescens* to split fat was confirmed by Berry (2).

Stark and Scheib (22) studied 486 cultures of lipolytic and caseolytic bacteria isolated from butter prepared and held under known conditions. Included were 40 cultures that resembled *Pseudomonas aeruginosa* in physiological properties; 30 of them produced a blue-green pigment, soluble in water, that turned dark brown with age and became red in the presence of acid. The remaining 10 cultures were identical with the 30 in all respects except that they produced a yellow pigment, slightly soluble in water, the color of which did not change in the presence of acid. This group was assumed to be a variant of *Pseudomonas aeruginosa*.

Storck (23) noted that alkali-forming bacteria constitute an important part of the milk flora during the winter months when the population of acid-forming bacteria is low. The *Bacterium fluorescens* group was one of the alkali-forming types present in raw milk, and three strains were isolated. These hydrolyzed fat and liquefied gelatin; two cultures coagulated milk before digestion, while the other culture digested milk without coagulation. Pasteurization at 63°C. for 30 minutes destroyed all the alkali-producing bacteria in milk except the spore-forming group.

Hansen (5) added 0.05 per cent of a milk culture of *Pseudomonas fluorescens* to milk used for cheese making and found that it did not significantly affect the flavor score or the nitrogenous decomposition in the cheese.

METHODS

DETECTION OF FLUORESCENT BACTERIA ON PLATES

Materials were examined for fluorescent bacteria by plating on beef infusion agar (pH 7.0 to 7.2) and incubating the plates 72 hours at 20° to 30°C., these conditions being favorable for production of the fluorescent pigment. In only a portion of the trials were fluorescent colonies on the plates evident from general observations, and plates regularly were examined under a relatively pure ultra-violet light in a dark room. Many of the fluorescent colonies were picked for purification and study.

ENRICHMENT PROCEDURES

Attempts to isolate fluorescent bacteria by direct plating of fresh milk and cream and products made from them seldom were successful because of the limited numbers of the organisms in such materials, and enrichment procedures were employed. With milk and cream these consisted of holding at 5° to 7°C. for some days and then plating.

Ice cream was allowed to melt at room temperature and plated soon after melting and again after holding at 5° to 7°C. for 5 days; with some samples, 11 ml. of the melted ice cream also was added to 99 ml. of sterile water and held at 5° to 7°C. for 5 days before plating.

Butter was plated directly in dilutions from 1:10 to 1:100,000. The enrichment procedure consisted of adding about 5 ml. of melted butter to a tube of litmus milk, shaking the tube, and plating after holding at 5° to 7°C. for 3 to 6 days.

Water samples were plated when received and also after holding at 5° to 7°C. for several days with about 5 per cent sterile milk added; the latter procedure was much the more effective. Such miscellaneous substances as feed, manure, and soil were examined by placing small portions in bottles of sterile water and plating after holding at 5° to 7°C. for a few days.

It is probable that enrichment attempts were not always successful and that fluorescent organisms sometimes were overgrown. Also, it is probable that even with enrichment the organisms were not obtained from all samples containing them because they could not always be detected on plates. Overcrowding of plates with non-fluorescent types tended to obscure fluorescent colonies since sometimes they were not observed on badly crowded plates but were noted on plates poured with higher dilutions of the sample. Deep subsurface colonies were not fluorescent because of an insufficient oxygen supply for pigment production; thin layers of agar in the plates overcame this difficulty to some extent.

EXPERIMENTAL

PRESENCE OF FLUORESCENT BACTERIA IN DAIRY PRODUCTS

MILK. Of 274 lots of milk delivered to plants in Iowa and Missouri, 178 (65.0%) yielded fluorescent organisms. Plates poured with some of the samples after enrichment contained mostly fluorescent organisms; the off flavors in such samples were stale, bitter, nutty, and rancid. Thirty-

five bottles of raw milk from the same areas yielded the organisms in 18 (51.4%) instances.

Fluorescent organisms also were isolated from 11 (44.0%) of 25 bottles of pasteurized milk from various dairies in the two states. After enrichment one sample contained mainly fluorescent organisms. Since the fluorescent bacteria are not heat resistant, they presumably were added from the equipment, etc., after pasteurization.

In connection with an investigation on mastitis, 580 samples of milk drawn aseptically from the individual quarters of 145 cows in four dairy herds were plated directly on beef infusion agar and the plates incubated 48 hours at 37°C. Fluorescent colonies were obtained from one or more quarters of four of the cows in numbers ranging from 500 to 4,500 per ml. The organisms isolated were identified as *Ps. aeruginosa.*

RAW SWEET CREAM. Of 149 lots of sweet cream delivered to plants in Iowa and Missouri, 87 (58.4%) yielded fluorescent organisms. After enrichment the organisms greatly predominated in several of the samples; the off flavors in such samples were unclean, bitter, cheesy, putrid, and rancid.

RAW SOUR CREAM. Fluorescent bacteria were obtained from 115 (77.7%) of 148 lots of sour cream, most of which were collected in Missouri. Of 104 lots examined earlier without enrichment, only 5 (4.8%) yielded the organisms. The acidities of the samples were not determined but varied from slightly sour to high acid.

ICE CREAM. Thirty-eight lots of ice cream from commercial plants and counter freezers in several states yielded fluorescent bacteria in 7 (18.4%) instances. The comparatively high sugar contents may have prevented any relative increases in the fluorescent organisms during enrichment.

FRESH, SWEET CREAM BUTTER. One hundred and thirteen samples of fresh, sweet cream butter were obtained from Iowa creameries at intervals during January to May, inclusive. Most of the samples were unsalted, and these were made both with and without butter culture; all were of high quality and except in a few instances scored 38 on flavor. Fluorescent organisms were isolated from 39 (34.5%) of the samples.

OTHER BUTTER. Of 72 samples of butter of miscellaneous types from Iowa, Missouri, Nebraska, and Oklahoma, 20 (27.8%) yielded fluorescent bacteria. Seven of the samples were farm butter and 2 (28.6%) contained fluorescent organisms. There were 24 samples of stored, unsalted, sweet cream butter that showed certain defects; of these 7 (29.2%) yielded fluorescent species. The remaining 41 samples were from sour cream; 22 were unsalted and some were prepared with use of butter culture. Eleven (26.8%) of the samples yielded fluorescent organisms.

PRESENCE OF FLUORESCENT BACTERIA IN MISCELLANEOUS MATERIALS

Fluorescent bacteria were found in 47 (95.9%) of 49 samples of water collected from pasture ponds, roadside pools, streams, rivers, farm

wells, and the roof of a building; also in 9 (75.0%) of 12 municipal water supplies. They were isolated from 15 (93.8%) of 16 samples of miscellaneous materials from a dairy barn and surroundings which included dust from the air and floor, cow feces, bedding, soil, alfalfa hay, beet pulp, cotton seed meal, linseed meal, ground corn, mixed grain, wheat bran, green wheat, green barley, and leaves of crab and hawthorne trees; one sample of each of the materials was examined, and all but one, the sample of linseed meal, gave positive findings.

GENERAL CHARACTERS OF FLUORESCENT BACTERIA ISOLATED

Of the cultures of fluorescent bacteria obtained from dairy products and miscellaneous sources, 496 were studied in some detail. They regularly were gram-negative, non-spore-forming rods, with polar flagellation, which grew well on the common media. In broth all the cultures grew at 7°C. and most of them grew at 3°C. All grew at 32°C.; a considerable number failed to grow at 37°C., although a few grew at 45°C.

Many of the cultures could not be identified on the basis of published descriptions.[2] All of them were placed in the genus Pseudomonas, although it is possible some of them would have been placed in the genus Phytomonas if pathogenicity for plants had been considered. The cultures which could be identified with considerable certainty showed minor variations within a species which indicates that this point must be considered in the preparation of descriptions of the organisms.

ACTION OF FLUORESCENT BACTERIA ON MILK

The changes produced in tubes of litmus milk at 21°C. by the 496 cultures of fluorescent bacteria were of six general types as follows:

a. Rapid digestion (usually complete within 5 to 10 days) from the top down, without noticeable coagulation and with formation of putrid odor and white or yellowish-white sediment; digested portion usually wine colored at first, later becoming light or dark amber, purple or green, and sometimes viscous.

b. Same as (a) except that digested portion is amber colored, non-viscous, and the odor suggestive of indol.

c. Alkaline reaction develops slowly, with no apparent proteolysis and no odor; bluish-gray color and white sediment after 14 to 21 days.

d. Alkaline reaction develops slowly, with slight digestion and slight putrid odor after 14 to 21 days; color bluish-gray to gray and some reduction after 21 to 28 days.

e. Slightly alkaline reaction after 7 days, followed by an acid reaction, but usually no coagulation after 21 days; no reduction except at bottom of tube.

f. Acid ring forms and later acid coagulation from the top down; slight proteolysis and May apple odor sometimes evident.

[2] A classification of the fluorescent organisms in dairy products and related materials is at present being developed.

Although each of the groups probably represents more than one species, the grouping appears to be a logical basis from which to work out a classification of the fluorescent organisms common in dairy products.

The action of the cultures on fat was tested with corn oil and cottonseed oil, rather than butterfat, because of the convenience of a relatively low melting point fat. Of the 496 cultures, 305 were lipolytic with the nile blue sulfate technic (13). The lipolytic cultures varied considerably in activity. All the cultures which hydrolyzed corn oil also hydrolyzed cotton seed oil; with a few cultures there were slight differences in the extents to which the two fats were hydrolyzed.

The effect of the fluorescent organisms on the flavor of milk and cream held at 5°C. was studied with 27 representative cultures. Of these, 15 digested litmus milk, 7 developed an alkaline reaction without digestion, and 5 produced acid coagulation; 9 of the proteolytic and 1 of the acid-coagulating cultures were lipolytic. The cultures were inoculated into sterilized skim milk and pasteurized (65.6°C. for 30 minutes) whole milk and cream (18% fat); an uninoculated sample of each product was held as a control.

During 18 days at 5°C. each organism produced essentially the same flavor in the three products, except that a slight nutty flavor was evident in some milk and cream cultures but not in the skim milk cultures. With the amount of inoculation used (0.1 ml. of a 24-hour milk culture per 100 ml.), off flavors were not noted until after 10 days in whole milk and cream although they sometimes were evident after 8 days in skim milk. Of the 15 proteolytic cultures, 11 developed a bitter flavor that usually became astringent or quinine-like as holding continued, 2 formed a nutty flavor, while the other 2, probably because of their slow growth, had no effect. One of the acid-coagulating cultures produced a putrid flavor after 12 days, but the other 4 acid-coagulating cultures and the 7 alkali-forming cultures developed only a slight stale flavor in milk and cream after 14 to 16 days. Although 10 of the organisms were lipolytic and 3 of them developed a rancid flavor in butter at 1° to 3°C., none of them produced a rancid flavor in milk or cream.

The protein breakdown in skim milk was studied with four actively proteolytic cultures and with one acid-coagulating culture, using the procedure employed by Long and Hammer (12). At 21°C. the proteolytic cultures greatly increased the soluble nitrogen in the milk; proteolysis was extensive after 2 days and increased regularly throughout the 10-day holding period. The degree of proteolysis varied somewhat with the different cultures. The acid-coagulating culture did not increase the soluble nitrogen during the 10 days. At 5°C. the soluble nitrogen in the milk was not increased significantly after 5 days by any of the cultures, but it was markedly increased after 14 and 21 days with three of the proteolytic cultures.

At 21°C. the distribution of nitrogen in the various fractions was essentially the same with the four proteolytic cultures. Along with the increase in total nitrogen there were increases in amino nitrogen and

in the nitrogen soluble and insoluble in the various reagents except that insoluble in trichloroacetic acid. The amounts of nitrogen in the fractions increased as the incubation period increased, but there was little change in the relationships of the various fractions although the amino nitrogen was fairly constant during the first 5 days and showed a pronounced increase after 10 days. At 5°C. the distribution of nitrogen was much the same as at 21°C., but the amounts were much less. Ammonia nitrogen was not increased during 21 days at 5°C. by any of the organisms.

ACTION OF FLUORESCENT BACTERIA ON BUTTER

The general action of 52 selected cultures of fluorescent bacteria on butter was studied as follows: 500 ml. of sterilized cream to which 10 ml. of a milk culture of a test organism had been added was churned, washed with sterile water, and worked in sterile equipment; part of the butter was left without salt, while 2 per cent salt was added to the remainder. With each organism, unsalted butter also was made with addition of 10 per cent butter culture to the cream. All the lots of butter were held in sterile petri dishes at 21°C. and changes in flavor noted during 7 days; the unsalted butter made without butter culture also was held at 1° to 3°C. and changes in flavor noted during 28 days. Acidity of the fat (3) in the unsalted samples made without butter culture was determined at once and after 7 days at 21°C. The data are given in Table 1.

In unsalted butter made without butter culture and held 7 days at 21°C., 47 (90.4%) of the 52 cultures produced some flavor defect, the off-flavors usually being evident after 2 days but sometimes not until near the end of the holding; at 1° to 3°C., off flavors were produced by 21 cultures during the 28 days of holding. Addition of butter culture to the cream or salt to the butter prevented development of flavor defects in the butter at 21°C. for 7 days by some organisms but not by others; with butter culture, 36 (69.2%) of the 52 cultures were detrimental to the flavor of the butter, while with salt 25 (48.1%) cultures produced flavor defects. Off flavors did not develop as rapidly, and commonly were not as pronounced, when butter culture or salt was used as without such additions.

In the unsalted butter made without butter culture and held at 21°C., more than one off flavor sometimes was evident in a sample, and the flavors present often changed as the sample was held. Cheesy or slightly putrid flavors were present in some samples when fairly fresh, but upon further holding only a rancid flavor was evident. Samples which developed a pronounced putrid flavor, however, continued to show this defect throughout the holding period, even when rancidity was evident later. Putrid or putrid and bitter flavors were quite conspicuous in some samples and were very offensive. Some cultures that hydrolyzed corn oil and cotton seed oil and increased the acidity of the fat developed only a putrid flavor in the butter; presumably, the flavor of the free fatty acids was submerged by the pronounced putrid flavor. A rancid flavor was quite intense in the 13 samples that developed only this type of

TABLE 1

ACTION OF SELECTED CULTURES OF FLUORESCENT BACTERIA ON BUTTER

Culture No.	Flavor of Butter After Holding 7 Days at 21°C.				Acidity* of Fat of Butter Made Without Butter Culture or Salt	
	No Butter Culture or Salt	10% Butter Culture in Cream	2% Salt in Butter	28 Days at 1° to 3°C.; No Butter Culture or Salt	Original	After 7 Days at 21°C.
1	putrid	sl.† putrid	good	good	0.70	2.20
2	putrid, rancid	sl. putrid	good	sl. putrid	0.70	3.40
3	sl. putrid	unclean	good	good	0.70	0.75
4	cheesy	sl. rancid	good	good	0.70	1.30
5	putrid, rancid	sl. putrid	good	good	0.70	3.10
6	cheesy	unclean	good	good	0.70	1.60
7	putrid, rancid	sl. putrid	good	good	0.70	2.70
8	rancid	sl. rancid	sl. rancid	sl. rancid	0.80	7.60
9	sl. putrid	good	good	good	0.80	1.00
10	putrid	putrid	sl. putrid	sl. putrid	0.80	2.30
11	rancid	rancid	rancid	sl. rancid	0.80	4.40
12	putrid	putrid	putrid	sl. putrid	0.80	2.50
13	putrid	good	good	good	0.80	0.80
14	rancid	sl. rancid	sl. rancid	sl. rancid	0.33	4.60
15	unclean	good	good	good	0.33	0.35
16	fruity, rancid	good	sl. putrid sl. rancid	sl. putrid sl. rancid	0.33	1.65
17	putrid, fruity	unclean	unclean	unclean	0.33	0.35
18	unclean, fruity	unclean	unclean	good	0.33	0.60
19	rancid	sl. rancid	sl. rancid	rancid	0.33	10.35
20	rancid	rancid	rancid	rancid	0.33	16.90
21	rancid	sl. rancid	sl. rancid	rancid	0.33	8.40
22	fruity	fruity	fruity	good	0.80	0.90
23	putrid	good	good	sl. putrid	0.80	2.70
24	rancid	sl. rancid	sl. rancid	rancid	0.80	10.60
25	rancid	good	good	good	0.80	3.00
26	rancid	sl. rancid	sl. rancid	rancid	0.80	12.00
27	rancid	rancid	good	good	0.33	4.00
28	cheesy, rancid	good	good	good	0.33	2.30
29	fruity, rancid	fruity, rancid	good	good	0.33	1.05
30	good	good	good	good	0.33	0.54
31	fruity, putrid, rancid	putrid, rancid	sl. putrid, sl. rancid	sl. rancid	0.33	1.95
32	good	good	good	good	0.33	0.30
33	putrid, fruity	sl. putrid	sl. putrid	fruity	0.33	0.32
34	putrid, rancid	putrid, rancid	rancid	good	0.43	3.75
35	putrid, bitter	good	sl. rancid	good	0.43	0.95
36	rancid	rancid	rancid	rancid	0.43	11.95
37	good	good	good	good	0.43	0.45
38	good	good	good	good	0.43	0.45
39	good	good	good	good	0.43	0.55
40	fruity	fruity	good	good	0.43	0.45
41	putrid	good	good	good	0.43	0.65
42	putrid, rancid	sl. bitter	good	sl. putrid, sl. rancid	0.43	1.70
43	putrid, bitter	sl. putrid	sl. rancid	sl. rancid	0.43	2.70
44	putrid, bitter	sl. putrid	sl. putrid	good	0.43	0.45
45	putrid, bitter	putrid	putrid	putrid	0.43	0.45
46	putrid, bitter	putrid	putrid	good	0.40	0.50
47	putrid, rancid	sl. rancid	good	putrid, rancid	0.40	2.20
48	rancid	sl. rancid	sl. rancid	good	0.40	5.20
49	rancid	sl. rancid	sl. rancid	good	0.40	2.70
50	putrid, bitter	sl. putrid	good	good	0.40	1.40
51	sl. cheesy	good	good	good	0.40	0.80
52	unclean	good	good	good	0.40	0.40

* = Ml. 0.1 N KOH required to neutralize 10 gm. fat.

† sl. = slightly.

defect and was correlated with a high acidity of the fat. A fruity flavor occurred in several samples along with other flavors, but 2 samples developed only this flavor. At the end of the holding the flavors in the samples were putrid, cheesy, or unclean, 13; putrid and bitter, 6; putrid or cheesy and rancid, 7; rancid, 13; fruity and rancid, putrid or unclean, 6; and fruity, 2.

The acidity of the fat was below 1.0 in 20 samples (none of which were rancid), between 1.0 and 2.5 in 13 samples (6 of which were rancid), between 2.6 and 5.0 in 11 samples (9 of which were rancid), between 5.1 and 10.0 in 3 samples (all of which were rancid), and above 10.0 in 5 samples (all of which were rancid).

One culture produced a bluish-purple color on the surface of unsalted butter within 5 days at 21°C. The color darkened somewhat during the holding, becoming bluish-black after 2 to 3 weeks. The coloration did not extend more than 2 to 3 mm. into the butter, but the entire surface of the butter in the container was affected. Another culture developed a salmon-pink color in unsalted butter within 1 week at 21°C.; it extended throughout the entire butter mass and did not change as the sample was held.

KEEPING QUALITY TESTS ON FRESH, SWEET CREAM BUTTER YIELDING FLUORESCENT BACTERIA

The 113 samples of fresh, sweet cream butter which were examined for fluorescent bacteria (p. 369) were held at 21°C. and the flavor noted at 2-day intervals for 6 to 8 days. In the original examination of the samples, 39 (34.5%) yielded fluorescent organisms, 22 directly and 17 only after enrichment. At the end of the holding the samples which originally had not yielded fluorescent bacteria were again examined for them; the results were largely negative, and the samples were not considered further.

Of the 39 samples which yielded fluorescent organisms, 19 (48.7%) developed defects at 21°C.; of the 74 samples not yielding such organisms, 26 (35.1%) developed defects. In each group of samples both slight and conspicuous defects were encountered. Among the conspicuous defects in each group cheesiness and rancidity were common; often cheesiness was first evident and then rancidity appeared. Of the samples yielding fluorescent organisms, only one showed a pronounced cheesiness unaccompanied by rancidity; in one sample most of the 27,500,000 non-butter culture organisms per ml. originally present were fluorescent and this butter rapidly became cheesy and rancid at 21°C.

DISCUSSION

The wide distribution of fluorescent organisms in dairy products is probably explained by their frequent presence in water, feeds, soil, and barn surroundings, from which they gain entrance to the products or to utensils and equipment coming in contact with them.

The conspicuous action of many of the organisms on dairy products

makes them potential causes of objectionable conditions in these materials; such action involves both the protein and fat. Because of the low growth temperatures of most of the organisms, they can develop at temperatures which might be expected to prevent bacterial action for rather extended periods. It appears that butter is the most susceptible of the common dairy products to serious spoilage by the fluorescent bacteria. Often it is held for rather extended periods at temperatures above the freezing point. The fluorescent bacteria can produce various types of defects in butter, but in agreement with their action on protein and fat, cheesiness and rancidity are especially common. However, both butter culture and salt tend to delay or prevent growth of the organisms.

The frequency with which rancidity, either alone or with some other off flavor, developed in butter made from cream inoculated with fluorescent organisms emphasizes the lipolytic action of these types. With two off flavors present, rancidity frequently is the more prominent because it is so objectionable.

The results of the keeping quality tests on the fresh, sweet cream butter indicate that defects can develop whether or not fluorescent organisms are in the butter; they also show that either with or without fluorescent organisms cheesiness and rancidity are especially prominent defects.

The failure to identify many of the cultures on the basis of published descriptions shows that an adequate classification of the fluorescent, gram-negative, non-spore-forming, rod-shaped bacteria so widely distributed in dairy products is greatly needed. It appears that the changes in litmus milk are a logical basis from which to develop such a classification.

SUMMARY

By means of enrichment procedures fluorescent bacteria were found widely distributed in the milk, cream, ice cream, and butter, and also in water and materials from dairy and barn surroundings. Such procedures were much more satisfactory for the detection of fluorescent organisms than direct plating.

The 496 cultures of fluorescent bacteria studied in some detail were gram-negative, non-spore-forming rods, with polar flagellation, which grew well on the common media and were psychrophilic. They produced six general types of changes in litmus milk; these may serve as a basis from which to develop a classification of the fluorescent organisms common in dairy products. Some of the cultures were actively proteolytic and some were not. Many of them hydrolyzed fat.

Selected cultures produced various types of off flavors in milk and cream held 18 days at 5°C., but rancidity was not detected although the selected cultures included some that were lipolytic.

Of 52 selected cultures, 47 (90.4%) produced flavor defects in unsalted butter held 7 days at 21°C. Addition of butter culture to the cream or salt to the butter prevented development of flavor defects by some organisms but not by others; with butter culture 36 (69.2%) of the 52 cultures produced off flavors in the butter, while with salt 25 (48.1%)

cultures produced off flavors. More than one off flavor sometimes was present in a sample, and the flavor often changed as the butter was held. Putrid and rancid flavors were especially common among the off flavors developed.

Of 113 samples of fresh, sweet cream butter (mostly unsalted), 39 (34.5%) yielded fluorescent bacteria, and 19 (48.7%) of these developed flavor defects in keeping quality tests; of the 74 samples not yielding fluorescent organisms, 26 (35.1%) developed defects. Among the conspicuous flavor defects in each group, cheesiness and rancidity were common; often cheesiness was first evident and then rancidity developed.

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A MICROSCOPIC METHOD OF STUDYING SOIL STRUCTURE

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INTRODUCTION

In soil studies employing the microscopic examination of thin sections of soil prepared (3, 5, 6) from representative soil samples, it is desirable to record the microscopic images for future reference and for comparison purposes with other soil samples of various soil types. Such recordings can be made by taking photomicrographs (2, 4, 6, 8) of the thin sections of soil by the usual photographic means (1). However, photomicrography requires the purchase of expensive equipment and the employment of considerable skill and knowledge of photography in the taking of good photographs (1).

In an attempt to record microscopic images of soil quickly and easily, a microprojection apparatus was devised which made possible the tracing of projected microscopic images of soil in the natural structural state as they appear in thin sections. The speed and simplicity of tracing the outlines of pore spaces directly from a projected microscopic image make such a method suitable for wide application.

This qualitative procedure contributes to the quantitative comparisons of pore spaces on the basis of size distribution and volume. Tracings of thin sections make available to others representative images of certain characteristics which are impossible to describe by words alone. However, it is realized that such tracings are not as instructive as actual examination of the object at first hand. The selection of a truly representative specimen of soil in thin sections requires a true concept of the nature of the population represented by the sample. Even without a microscope it is possible to get a macroscopic idea of the quality of the structure of a soil by looking at a well-made thin section. For the best observation, some kind of illumination should be used, such as laying the thin section on the face of the glass of a substage microscope lamp.

DESCRIPTION AND USE OF THE MICROPROJECTION APPARATUS

The microprojection apparatus is shown in Figure 1. A Spencer petrographic microscope was used in producing the images. Since each Nicol prism wastes about 50 per cent of the light entering it (1), a Zeiss microprojector equipped with a carbon arc was employed for the source of light and for projecting the images onto a ground-glass plate. To facilitate the operation of the hand-fed carbon-arc lamp, the coaxial knobs used

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in adjusting the carbons were extended, making them easily accessible during the operation of the apparatus. A table was remodeled by replacing the top with a 20-inch square, ground-glass plate and attaching adjustable legs so that it could be raised or lowered for the purpose of varying the magnification of the projected image. Black cotton flannel cloth held in a conical form by spring steel wire served as a bellows. Exclusion of all stray light permitted the projection of microscopic images in a lighted room although the best images were produced in a dark room. The magnification of the projected image was checked with a stage micrometer graduated to 0.01 mm.

To insure that none of the distortion due to spherical aberration would be included in the microscopic fields traced, a brass washer was inserted in the section of the eyepiece adapted to hold the eyepiece micrometer. The solid part of the washer reduced the diameter of the microscopic field in the eyepiece sufficiently to eliminate the distorted edges. Because it was necessary to rotate the stage in order to differentiate between minerals and pore spaces (6), a bumper device was designed so that the rotating stage could be easily and accurately returned to its original position at the completion of a determination.

Well-prepared thin sections (5) of a thickness of 0.07 ± 0.01 mm. were used in which the outlines of the pore spaces stood out more distinctly than in thin sections of the 0.03 mm. thickness commonly employed for mineralogical studies. The mechanical stage was useful in locating the structure to be traced and in recording for reference purposes the location of the microscopic field for each thin section traced.

In order to obtain a distinct outline on tracing paper of the magnified image appearing on the ground-glass plate, a hard lead pencil was used in tracing the pore spaces. For ease of distinguishing pore spaces from soil minerals and the ground mass, a gypsum plate was inserted in the accessory aperature of the microscope. This caused the pore spaces to appear as a pink color under crossed Nicols and on rotation of the microscope stage, while the crystalline and non-crystalline materials exhibited their characteristic optical properties. From 10 to 20 minutes were required to trace the outlines of a projected microscopic image, depending on the number of pore spaces present and on the number of minerals which had to be distinguished from the pore spaces.

RESULTS AND DISCUSSION

Figures 3 to 8 inclusive show the relative sizes, shapes, and distributions of pore spaces for the profiles of the Marshall and Shelby silt loam soils from the Soil Conservation Experiment Stations at Clarinda, Iowa, and Bethany, Missouri, respectively (6). For comparative purposes these characteristics are also presented in Figure 2 in a graphical form from previously determined data (6). It is readily seen that the Marshall (Fig. 2) had a more porous structure throughout the profile than the Shelby. It is apparent that in the surface horizon (Figs. 3 and 4), the Marshall had the larger-sized pores and a greater total number of pores. At

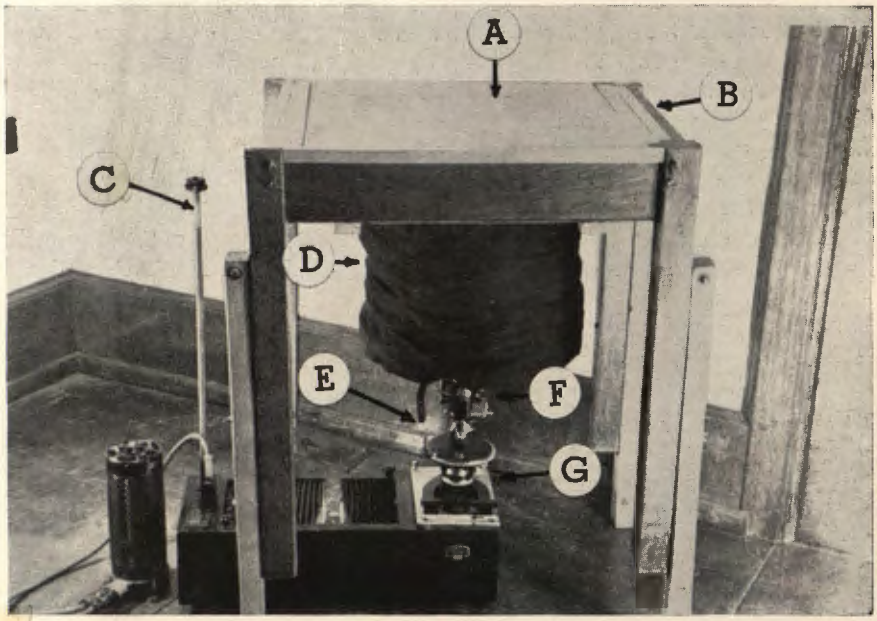

FIG. 1. Microprojection apparatus for tracing projected images of a microscope field. (A) ground-glass plate, (B) adjustable table, (C) coxial knob extension, (D) bellows, (E) bumper apparatus, (F) petrographic microscope, (G) microprojector.

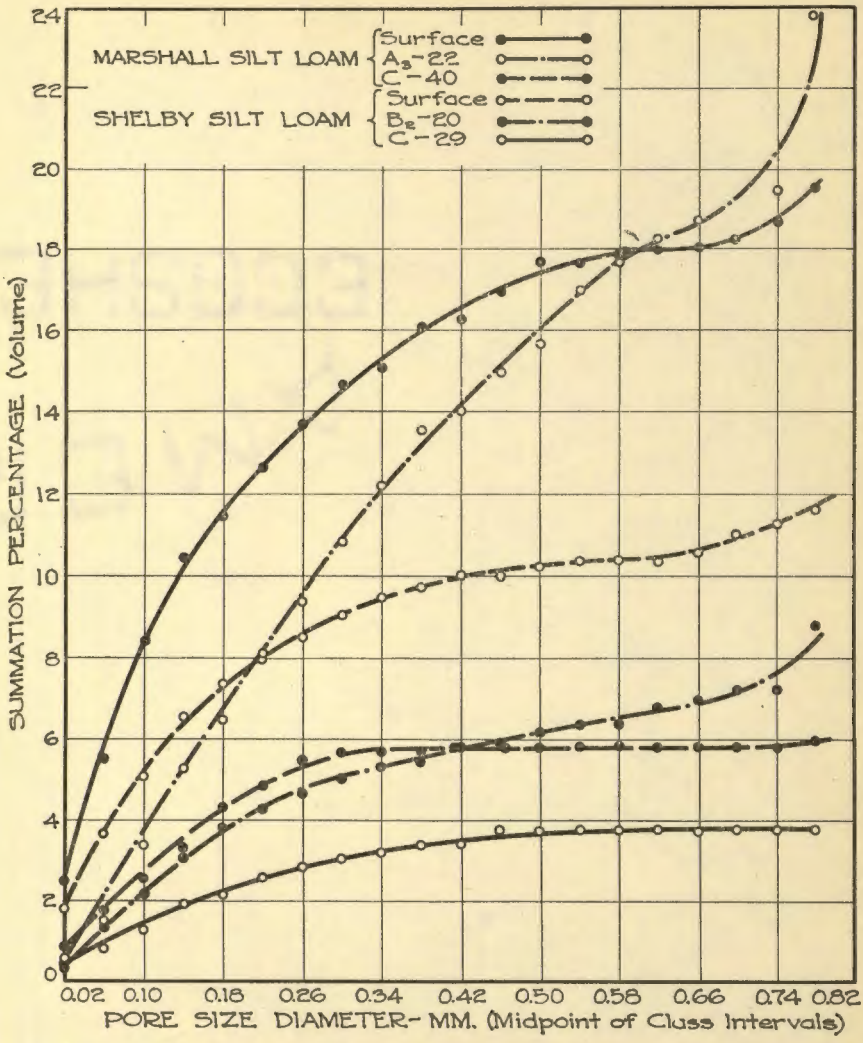

FIG. 2. Size distribution of pore spaces obtained by the micrometric method.

an approximate depth of 20 inches (Figs. 5 and 6), a striking difference is noted in the pore space relationships. The relatively numerous variable-shaped pores in the Marshall at this depth in comparison with the crack-like pore structure in the dense mass of the Shelby suggest a continuation with depth of a larger number of non-capillary pores in the Marshall than in the Shelby. In the C horizons (Figs. 7 and 8) but at different depths, it is evident that the amount of non-capillary pores in the Marshall still continued to be greater than in the Shelby.

A qualitative observation of the accuracy of micrometric analysis (7) is provided by a comparison of the data in Table 1 with Figures 9 and 10.

FIG. 3. Microprojection tracing. Surface horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 4. Microprojection tracing. Surface horizon, Shelby silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 5. Microprojection tracing. A_1–22 inch horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 ×.

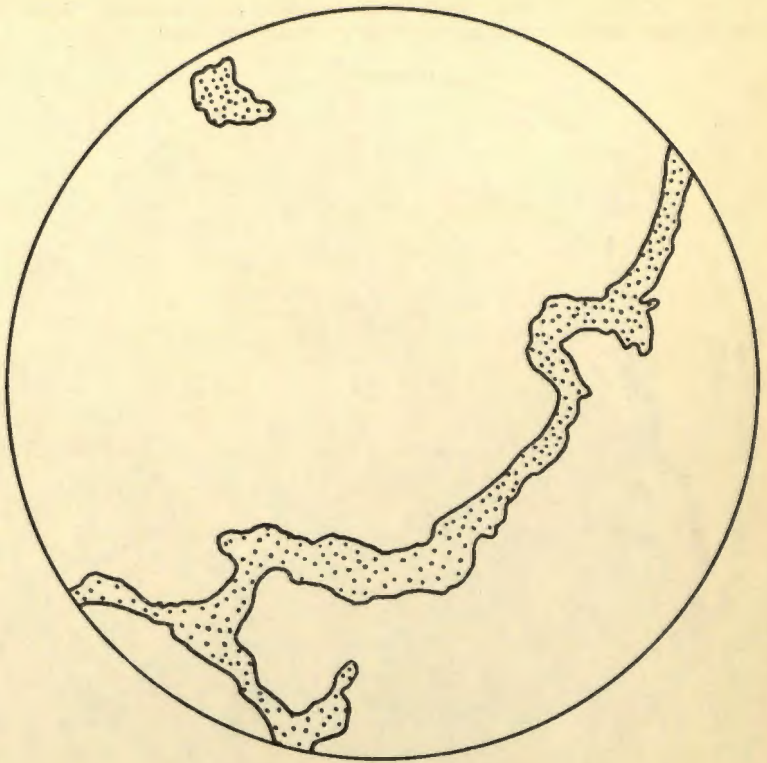

FIG. 6. Microprojection tracing. B_1–20 inch horizon. Shelby silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 7. Microprojection tracing. C–40 inch horizon, Marshall silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 8. Microprojection tracing. C–29 inch horizon, Shelby silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 9. Microprojection tracing. Surface horizon (virgin), Marshall silt loam. Stippled areas represent pore space. 44.5 ×.

FIG. 10. Microprojection tracing. Surface horizon (cultivated), Marshall silt loam. Stippled areas represent pore space. 44.5 ×.

TABLE 1

SIZE DISTRIBUTION OF PORES OBTAINED BY THE MICROMETRIC METHOD

MARSHALL SILT LOAM*

MID POINT OF CLASS INTERVAL	MICROMETRIC METHOD	
	Virgin†	Cultivated†
mm.‡	%**	%**
0.096	1.36	0.81
0.146	1.38	0.78
0.205	1.36	0.60
0.263	1.01	0.54
0.322	0.73	0.41
0.380	0.62	0.31
0.439	0.51	0.46
0.497	0.23	0.05
0.556	0.30	0.09
0.614	0.38	0.14
0.673	0.27	0.00
0.731	0.29	0.05
0.790	0.21	0.06
0.848	0.23	0.06
0.907	0.25	0.06
0.965	0.20	0.00
1.024	0.07	0.00
1.082	0.15	0.00
1.141	1.41	0.08
Total.....	10.96	4.50

*Soil samples obtained near Cherokee, Iowa.

†Mean values obtained from four thin sections.

‡Pore diameters.

**Per cent of the total volume of the sample.

These figures are microprojection tracings of the structures exhibited by the virgin and cultivated samples of the Marshall silt loam soil obtained near Cherokee, Iowa. The wide differences in the total amounts of pore space, in the size distribution of the larger pores, and in the outlines of aggregates as suggested by the arrangements of the pore spaces for these two soils are apparent in these two tracings.

In none of the microprojection tracings for the soils studied did there appear to be any directional orientation of the pore spaces.

SUMMARY

1. An apparatus was devised which made possible the quick and easy tracing of projected microscopic images of soil in the natural state as they appeared in thin sections. It also facilitated the comparison of the larger-sized pore spaces (non-capillary range) on the basis of shape, size distribution, and volume.

2. Studies of the natural structure of the Marshall and Shelby silt loam profiles as revealed by tracings of projected microscopic images of thin sections of soil and by the micrometric analysis of pore space supplied information concerning their pore space relationships. The porosity

of the Marshall was much greater throughout the whole profile than that of the Shelby. Furthermore, the occurrence of large pores continued to a greater depth in the Marshall, there being many more large pores in the 22-inch sample (A_3) of the Marshall than in the 20-inch sample (B) of the Shelby.

3. The pore space relationships exhibited by microprojection tracings of a cultivated and virgin surface soil horizon were studied and compared with data obtained by the micrometric analysis of pore space. The results showed that the virgin soil had twice the volume of pores and a higher percentage of large pores than did the cultivated soil.

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A COMPARISON OF THE ALUMINUM-CHLORIDE AND THE SULFURIC-ACID METHODS FOR QUANTITATIVE ESTIMATION OF WOOL[1]

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For at least sixty-five years sulfuric acid of various concentrations has been recommended for quantitative estimation of the wool of cellulosic admixtures, and change in weight of wool by sulfuric acid has been reported by many investigators.

Bayer (3) immersed a half-gram sample of wool 12 hours in 400 volumes of a mixture, 4 volumes of concentrated sulfuric acid and 1 of water, then drained and treated the residual wool 5 hours with a second portion of the acid, and diluted the combined suspensions with 6 volumes of 1 : 1 ethanol before filtering, washing, and drying the residue at 100°C. This procedure resulted in a 2 per cent loss of wool.

Heermann (11) reported that 5 grams of wool which had been extracted with diethyl ether and with 96 per cent ethanol increased 2 per cent in weight when steeped 3 hours, and 1.5 per cent when steeped 6 hours, in 100 volumes of 80 per cent sulfuric acid in stoppered flasks before dilution, filtration, washing with dilute ammonium hydroxide, and drying to constant weight at 105° to 110°C.; Heermann further reported that the wool lost 5.5 per cent in weight when left in the acid for 24 hours.

Matthews (19) and Green (10) found that wool lost 2.5 per cent of its weight when treated for 12 hours in 1 : 1 sulfuric acid before dilution with 3 volumes of ethanol and water; 3 determinations by their method in this laboratory (34) resulted in a mean loss of 33.6 ± 4.3 per cent of wool.

Krais and Biltz (15) heated wool to 50°C. in 44 per cent sulfuric acid and allowed it to remain in the cooling bath for 24 hours; 4 determinations by their method in this laboratory (7) resulted in a mean loss of 31.7 per cent of wool. Lloyd and Priestley (18) used acid of this concentration for 20 minutes at 50°C. to remove cotton, and for 24 hours at 25°C. to remove regenerated cellulose from mixtures containing wool; in this laboratory (7) 6 determinations by the former method resulted in a loss of 1.5 per cent of wool and 6 by the latter method, but using 62 per cent sulfuric acid, yielded a loss of 35.8 per cent. A mean increase of but 0.04 per cent (from — 0.5 to + 0.1) for wool treated 3 hours with 80 per cent sulfuric acid according to Schulze's method has been reported by several analysts (29, 13, 14, 16, 17, 8, 12) although Viertel (31) obtained an 8

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TABLE 1

A COMPARISON OF THE ALUMINUM-CHLORIDE AND THE SULFURIC-ACID METHODS FOR QUANTITATIVE ESTIMATION OF WOOL

TREATMENT OF WOOL	PARALLEL DETERMINATIONS	RESIDUAL WOOL			
		Weight	Ash	Total Sulfur	Sulfate Sulfur
	Number	Percentage of Wool	Percentage of Wool	Percentage of Wool	Percentage of Wool
A. None	6	100.0	0.10 (0.01)*	3.76 (0.01)	0.30 (0.01)
B. Aluminum-chloride method	2	98.7 (0.4)	.....	.....	none
	6	98.8 (0.2)	.....	3.46 (0.03)	.....
	6	98.8 (0.4)	0.43 (0.15)	.....	.....
	6	100.1 (0.4)	.....	.....	none
	6	100.7 (0.6)	.....	3.43 (0.06)	.....
	6	101.0 (0.3)	1.04 (0.11)	.....	.....
C. Sulfuric-acid method	2	99.8 (0.4)	0.05 (0.01)	.....	.....
	6	100.4 (0.2)	.....	4.82 (0.07)	.....
	6	101.2 (0.2)	.....	.....	1.50 (0.07)
	6	101.4 (0.6)	.....	5.04 (0.15)	.....
	6	.....	.....	.....	1.51 (0.09)

*Mean deviations are within parentheses.

per cent and Schaeffer (28) a 10 per cent increase in weight for wool with this method.

Ryberg (27) reported gains of from 1.8 to 6.8 per cent for wool treated at 30°C. for 15 minutes with 70 to 85 per cent sulfuric acid. The use of 70 per cent sulfuric acid has been further modified (2b, 2c, 1a, 1b, 2d).

Yamada and Ikoma (33) and Schaeffer (28) favor the aluminum-chloride method (2a, 22, 23, 20, 24, 25, 26, 2c) described by Viertel (31) as giving results of close concordance and by Skinkle (30) as not so satisfactory.

Since our work was done, Weidenhammer, Prisley, and Ryberg (32) have reported an inter-laboratory comparison of the sulfuric-acid and the aluminum-chloride methods based on weight of residual wool. As reported in their study the change in weight of an all-wool fabric by the aluminum-chloride method varied from — 2.3 to + 1.4 per cent among 25 determinations and for the 5 sets of 5 determinations the means ranged from — 2.0 to 1.1 per cent; change in weight of this all-wool fabric by the sulfuric-acid method varied from — 3.6 to + 2.8 per cent among 25 determinations, and for the 5 sets of 5 determinations the means ranged from — 2.9 to + 2.4 per cent. Weidenhammer, Prisley, and Ryberg concluded that both methods were sufficiently accurate and precise to be useful but that, because it was more convenient and less time consuming, the sulfuric-acid method was to be preferred.

The aluminum-chloride and the 70 per cent sulfuric-acid methods are compared in this study by their effects on the weight, ash, total sulfur, and sulfate sulfur of the residual wools (Table 1).

EXPERIMENTAL PROCEDURE

PREPARATION OF WOOL

Plain-woven unscoured wool which contained neither sulfite sulfur (9) nor selenium (6) was cut for analysis and extracted continuously 20 hours with sulfur-free benzene in a Soxhlet extractor, dried in air at room temperature, washed in 100 volumes of 0.1 per cent saponin for 5 minutes at 40°C., rinsed 5 times in distilled water at room temperature, dried, again extracted continuously with benzene for 20 hours, dried, rinsed 8 times in water, and dried at room temperature (5).

ALUMINUM-CHLORIDE TREATMENT OF WOOL

A 5-gram sample of this wool was dried until constant at 105° to 110°C. and immersed 10 minutes in 40 volumes of a boiling solution, 9 grams of aluminum-chloride hexahydrate per 100 milliliters of water. The residue was drained, dried at 105° to 110°C. for 2 hours, rubbed thoroughly in an attempt to remove all the powdery precipitate, rinsed in 100 volumes of dilute hydrochloric acid (1 part by volume of concentrated hydrochloric acid diluted with 9 parts of water), washed free of chloride, dried at room temperature (2c), and then until constant at 105° to 110°C. before analysis for ash, total sulfur, and sulfate sulfur. Six parallel

blank determinations by this aluminum-chloride method yielded a residual wool, 0.07 ± 0.01 per cent ash and 99.9 ± 0.2 per cent the weight of the original wool.

SULFURIC-ACID TREATMENT OF WOOL

A 5-gram sample of wool was dried until constant at 105° to 110°C., immersed 10 minutes in 100 volumes of boiling sulfuric acid (1 per cent hydrogen sulfate by weight), drained, worked 15 minutes at 38°C. in 100 volumes of sulfuric acid (70 per cent hydrogen sulfate by weight), drained, washed with cold water, immersed in 2 per cent sodium hydrogen carbonate at room temperature for 5 minutes, washed free of sulfate, and dried first at room temperature and then at 105° to 110°C. until constant in weight before analysis for ash, total sulfur, and sulfate sulfur.

ASH

A 5-gram sample of wool was placed in a porcelain crucible and ignited until constant at the red heat of an electric furnace, 600° to 700°C.

TOTAL SULFUR

A 5-gram sample of wool was dissolved in 100 milliliters of a solution, 1 part nitric acid and 2 parts water, in a covered casserole on a steam plate. After the addition of 100 milliliters of Benedict-Denis reagent (4) the mixture was evaporated to dryness, heated to dull red for 10 minutes, dissolved in 100 milliliters of 10 per cent hydrochloric acid, and filtered. This filtrate was diluted to 375 milliliters and boiled during the dropwise addition of 25 milliliters of 10 per cent barium chloride. The precipitate was digested 15 hours on a steam bath, filtered into a weighed Gooch crucible, washed free of chloride, dried in an oven at 105° to 110°C., and ignited at 600° to 700°C. in an electric muffle furnace for 25-minute periods until of constant weight. Blank determinations were made with the reagents.

SULFATE SULFUR

A 5-gram sample of wool was dissolved in 50 milliliters of 30 per cent hydrochloric acid on a boiling water bath, cooled, diluted with 50 milliliters of water, and filtered. The filtrate was brought to boiling for precipitation of its sulfate as described before.

SUMMARY

1. The aluminum-chloride and the 70 per cent sulfuric-acid methods for estimation of the wool of cellulose admixtures have been compared by their effect on the weight, ash, total sulfur, and sulfate sulfur of the residual wool.

2. The change in weight of wool by the aluminum-chloride method varied from — 2.1 to + 1.9 per cent among 32 determinations; for 5 sets of 6 parallel determinations the means ranged from —1.2 to +1.0 per cent. The sulfate sulfur of the wool was removed by this method and the ash in-

creased from 0.10 ± 0.01 to 0.43 ± 0.15 per cent for one set and to 1.04 ± 0.11 for another set of parallel determinations.

3. The change in weight of wool by the sulfuric-acid method varied from — 0.6 to + 2.4 per cent among 18 determinations; for 3 sets of 6 parallel determinations the means ranged from 0.4 to 1.4 per cent. The sulfate sulfur of the wool was increased from its original 0.30 ± 0.01 to 1.51 ± 0.08 per cent, the total sulfur from 3.76 ± 0.01 to 4.82 ± 0.07 for one set, and to 5.04 ± 0.15 for another set of 6 parallel determinations although the ash was lowered from 0.10 ± 0.01 to 0.05 ± 0.01 per cent.

4. With the proportion of wool at a maximum, these data describe the 70 per cent sulfuric-acid method as the better of the 2 methods.

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NEW ANTS FROM MINNESOTA, IOWA, AND WISCONSIN

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Iowa, Minnesota, and northern Wisconsin are regions which seem to have been neglected by American myrmecologists. Thus it is not surprising that new ants, especially parasitic species which tend to be rare, sporadic, or circumscribed in distribution, can be collected in these regions.

Holotypes and most of the paratypes are to remain in the author's collection. A few paratypes of each species will be deposited in the National Museum and the Iowa State College collection. There are also a few paratypes in the collection of Herbert T. Dalmat of New York City, who accompanied the writer on the collecting trip during which most of these species were taken.

Formica reflexa, n. sp.

WORKER

Length, 4.65–5.27 mm.

Head, excluding the mandibles, a little longer than broad, somewhat broader behind than in front, with convex posterior margin, evenly and broadly rounded posterior corners, and moderately convex sides. Mandibles 7-toothed. Maxillary palpi approximately .64 mm. long. Clypeus feebly carinate, subangularly produced in front. Apex of frontal area indistinct. Frontal carinae short, about 1½ times as long as the diameter of the antennal foramina, evenly diverging behind. Eyes hairy. Funicular joints 2–10 subequal in length, the penultimate never shorter than the second. Pro- and mesonotum moderately convex in profile, the middle of the mesonotum straight. Epinotum rather angulate in profile, the base and declivity nearly straight and subequal in length. Petiole small, anterior surface convex, the posterior surface less so; superior border unusually blunt, approaching *Polyergus* species in this respect; the sides and superior border at least slightly convex when seen from behind. Entire body more slender and less robust than *F. dakotensis*.

Head, legs, and gaster feebly shining, clypeus and thorax subopaque. Shagreening on head, thorax, and gaster fine and dense, concentrically set on the pro- and epinotum.

Erect hairs sparse; rather short on most parts of the body, particularly on the pro- and mesonotum where they are about .03–.05 mm. in length. An occasional hair slightly clavate. No erect hairs on the scapes, femora, or tibiae, few on the front and mesonotum, sparse on the dorsum of the gaster. Pubescence extraordinary, consisting of fine hairs almost as long as some of the erect hairs, often growing out from the surface suberectly but sharply reflexed in the middle so that the distal ends strike the surface. Reflexed pubescence most obvious on the gaster.

Pubescent hairs numerous but not dense, nowhere concealing the surface, the distance between the bases of adjacent hairs about .03 mm.

Head and thorax reddish brown, the vertex, occiput, pro- and mesonotum sometimes feebly infuscated. Gaster black to the naked eye, under magnification often deep brown with black posterior borders to the segments. Antennae nearly black, legs dark brown.

FEMALE

Length, 5.41–6.32 mm.

Head, excluding the mandibles, as broad as long (about 1.14 mm.), broader behind than in front, with the posterior border convex except in the middle where it is slightly excised, the posterior corners evenly rounded, and the sides nearly straight. Mandibles 7-toothed. Clypeus ecarinate or nearly so. Frontal area rather indistinct. Frontal carinae diverging behind; short, about 1½ times as long as the diameter of the antennal foramina. Funicular joints 2–10 subequal in length, the penultimate joint as long as or slightly longer than the second, and only slightly shorter than the fourth. Eyes hairy, little or no larger than in the worker. Thorax five-sixths as wide as the head, approximately .93 mm. across the wing insertions. In profile the pronotum and epinotum more sloping than in *F. dakotensis*. Petiole low and even blunter than that of the worker, the sides and superior border moderately convex when seen from behind. Wings about 5.5 mm. long. Whole body much more slender and less robust than *dakotensis*.

Entire body very shining, without shagreening, marked only by the punctures from which the pilosity and pubescence arise.

Erect hairs not very numerous on any part of the body, most abundant on the dorsum of the thorax, sparse on the head and gaster. Hairs on the dorsum of the thorax often slightly clavate. Pubescence unique, consisting of fine hair a little shorter than the erect hairs, growing out from the surface suberectly, but sharply reflexed in the middle so that the distal ends strike the surface. Reflexed pubescent hairs numerous but not dense on all body surfaces, the bases of adjacent hairs about .03 mm. apart. Pubescent hairs flexuous on the legs but usually not sharply reflexed.

In mature specimens, head deep blackish brown, most of the thorax dark brown, the scutellum nearly black, the epinotum lighter. Gaster black to the naked eye. Legs dark brown. Antennae black. In dorsal aspect, the insect has a dark, almost black appearance.

MALE

Length, 6.54–7.49 mm.

Head, excluding the mandibles, distinctly broader than long, the posterior border broadly rounded. Mandibles indistinctly 3-toothed. Eyes hairy. Funicular joints 2–10 gradually decreasing in length, the second about one-fourth again as long as the penultimate, and as long as the first. Whole body more slender and less robust than in *F. dakotensis*. Petiole low and blunt, the superior border straight or slightly convex when seen from behind.

Head and thorax subopaque, gaster feebly shining.

Erect hairs short, numerous on the dorsum of the thorax, much sparser on the head and gaster. Pubescent hairs long, straight, but usually subappressed to suberect so that it is difficult to distinguish between pilosity and pubescence. Pubescence moderately sparse, nowhere concealing the surface; denser, however, than on the worker and female.

Color black, legs brown.

Described from 53 virgin females (most of them partially callow), 41 males, and 129 workers taken from a small, obscure, roadside nest near Hibbing, Minn., August 13, 1941. This species was also found at Owatonna, Minn. (23 virgin females and 33 workers, August 17, 1941, and 10 workers, July 6, 1940); at Jenkins, Minn. (24 workers and 9 males, August 11, 1941, and 18 workers, July 10, 1940); and at Spirit Lake, Iowa (20 workers from four small nests, June 12, 1940, and 5 workers, July 15, 1940).

Differing from all other species of *Formica* known to the writer by having the pubescence reflexed and the petiole unusually blunt. Among the species of *Formica*, *reflexa* seems most closely related to *dakotensis* Emery. *F. reflexa* differs from this species mainly by having the sides of the head less convex, reflexed rather than straight pubescence, smaller size, and the petiole very blunt rather than cuneate in profile and convex above rather than truncate or excised when seen from behind.

F. ferocula is described by Wheeler as having a very blunt petiole. This may possibly be as blunt as that of *reflexa*. *F. ferocula* differs from *reflexa* by having the head as broad as long, the posterior border of the head feebly excised instead of convex, the first four funicular joints longer than the penultimate, the epinotum with a slightly convex base and distinctly concave declivity rather than with a straight base and declivity, the erect hairs numerous on parts of the thorax and absent on the posterior portions of the head, and by not having the pubescence reflexed. *F. ferocula* was thought by Wheeler to be allied to the *ciliata* group of species. Thus it probably is not closely related to *reflexa*.

Although the females of *reflexa* are very small and definitely microgynous, this species does not seem closely related to any of the species of the *microgyna* group. *F. reflexa* can be distinguished from all members of this group by the unusual shape of the petiole, the reflexed pubescence, the proportions of the funicular joints, and the very small, rather uniformly sized worker cast.

The very small, uniform size of the worker, unlike most species of the *rufa* group, and the small, blunt petiole (as if atrophied) seem to show a highly developed parasitism.

Of the seven nests that the writer has found, none were without numerous workers of the host species, *F. fusca subsericea*, the *subsericea* workers in all cases outnumbering the *reflexa* workers at least several to one. This immediately suggests permanent social parasitism of the dulotic type, like that of *Polyergus* species. That *reflexa's* relationship with *subsericea* is not of the dulotic type is evidenced by the fact that *reflexa* lacks the clypeal notch characteristic of all species of *Formica* which engage in dulosis, and that the workers are much smaller, weaker,

and even more timid than their hosts. Upon opening their nests, the *reflexa* workers disappeared as quickly as possible, leaving the *subsericea* workers to defend the nests.

F. reflexa can hardly be a temporary social parasite. In this type of parasitism it is usually very difficult to find mixed colonies.

If *reflexa* is a permanent social parasite of the nondulotic type, the females should exhibit very strong inquilinous characteristics, for it would be fatal to seek adoption in an incipient or depauperate colony as do all other parasitic *Formica* species. Instead, adoption must be secured in a flourishing colony which can furnish enough host workers to rear *reflexa* broods for at least two or three seasons. However, if the host queen is retained as in *Strongylognathus testaceous* of Europe, incipient nests could be chosen.

Much experimentation should be done with this species to determine its exact relationship with *F. subsericea.*

Formica fossaceps, n. sp.

WORKER, MAXIMA

Length, 6.65–7.71 mm.

Head, excluding the mandibles, slightly longer than broad, narrower in front than behind, with feebly excised or straight posterior border and nearly straight sides. Clypeus evenly rounded in front, ecarinate or distinctly carinate only at the extreme front. Clypeal fossae strikingly deep; antennal fossae also rather deeply impressed. Maxillary palpi short. Apex of frontal area rounded. Frontal carinae diverging, about twice as long as the diameter of the antennal foramina. Frontal furrow indistinct, ending in a shallow pit level with the eyes. Eyes hairless. Scapes surpassing posterior lateral corners of the head by nearly two-fifths of their length. Funicular joints gradually decreasing in length toward the penultimate, the second approximately four-fifths as long as the first and about one-third again as long as the penultimate, the third slightly shorter than the second; joints 5–8 rather unusual in appearance, each narrow at the base but swollen to more than 1½ times the base width near the apex. To the naked eye these joints are somewhat bead-like.

Pro- and mesonotum not as convex as in *F. rufa obscuriventris.* Meso-epinotal depression not as deep. Epinotum in profile with subequal base and declivity, the base slightly convex, the declivity feebly concave. Petiole large, higher than the epinotum, in profile cuneate, the superior border very sharp. Seen from behind, the petiole broad, measuring up to .77 mm. wide, and strongly subangularly or convexly produced upward.

Head and legs moderately shining, gaster more shining but not as highly polished as that of the female, the shagreening on the gaster very fine and superficial. Thorax subopaque.

Erect hairs absent on most body surfaces, present on the dorsum of gaster near the apex, venter of gaster, venter of petiole, and coxae. Pubescence very short and sparse, especially on the head and gaster,

rather dense only on the base of gaster, petiole, epinotum, and appendages.

Head and thorax ferruginous, appendages brown, entire gaster deep black.

WORKER, MEDIA

Length, 5.23–6.62 mm.

Differing from the major in having the head straight or slightly convex behind, the clypeal fossae a little less deep, the basal funicular joints shorter in proportion to the penultimate, the middle joints less bead-like, and the head and thorax often infuscated.

WORKER, MINIMA

Length, 3.70–4.58 mm.

Differing from the major in having the head definitely longer than broad and slightly convex behind, the clypeus distinctly carinate, the clypeal fossae more normal in depth, funicular joints 2–10 more nearly subequal in length, and the middle joints not bead-like. Also differing in having the body a little less shining and the head and thorax heavily infuscated.

FEMALE

Length, 7.19–7.84 mm.

Although little shorter than the females of *F. rufa obscuriventris*, strikingly more slender and much less massive than this species.

Head, including the mandibles, triangular in shape, the outer borders of the mandibles nearly aligned with the sides of the head; excluding the mandibles, slightly longer than broad (about 1.38 mm. wide), one-third again as wide behind as in front, with straight posterior border and sides. Clypeus evenly rounded in front, ecarinate. Clypeal fossae extraordinarily deep, the middle part of the clypeus between them strongly convex in transverse section. Frontal carinae diverging, approximately twice as long as the diameter of the antennal foramina. Frontal furrow very short and indistinct, not reaching beyond the frontal carinae. Funiculi much as in the major worker, but joints 5–8 not as bead-like and the second joint only a little shorter than the first. Eyes hairless, a little larger than those of the worker. Thorax narrower than the head, about 1.2 mm. wide. Petiole large, cuneate in profile, with sharp superior border, subangularly produced upward when seen from behind.

Entire body, especially the gaster, glabrous and extremely smooth. Thorax a little less shining than the head and gaster. Shagreening on the gaster scarcely perceptible, the surface marked only by the very sparse, minute punctures from which the pubescence arises.

Pilosity entirely lacking on all dorsal regions of the body; some slender, scattered hairs on the venter of the gaster and coxae. Pubescence very fine, short, and sparse, about .01–.02 mm. in length, rather dense only on the base of the gaster and appendages; extremely sparse on the head.

Head and thorax light brownish red. Gaster entirely deep black except at the extreme base. Head lightly infuscated behind the eyes.

Mesonotum heavily infuscated in a median frontal spot and along the parapsidal sutures. Scutellum and metanotum black.

MALE

Length, 8.06 mm.

Head, excluding the mandibles, distinctly broader than long, much broader behind than in front. Mandibles feebly 3-toothed. Clypeal fossae very deep. Funicular joints 2–10 gradually decreasing in length, the penultimate a little over one-half as long as the second, the second one-fourth again as long as the first. Petiole in profile, with convex anterior and flat posterior surfaces; seen from behind, subrectangular, the superior border sinuate but nearly truncate, the sides almost straight.

Head and thorax mainly opaque, the declivity of the epinotum glabrous; gaster feebly shining.

Erect hairs nearly absent on the dorsal surfaces of the body, a few on the gula, profuse on the venter of the gaster toward the apex. Pubescence sparse, but longer and much denser than in the worker and female.

Color black, the antennae dark brown, legs light brown.

Described from 12 females, 93 workers, and 1 male taken from a nest in wooded pasture near Winterset, Iowa, June 27, 1941. This nest was located around the base of a bush, plant debris being used in the construction of a low dome. Immediately under this dome were the brood, workers, and sexual forms.

This species seems definitely related to *F. rufa* and its numerous subspecies and varieties. It differs from all of them, however, by the paucity of both pilosity and pubescence, the very shining surface, and the depth of the clypeal fossae. *F. rufa obscuriventris* seems to be most closely related in the structure of the clypeal fossae and the funiculi. *F. fossaceps* can be distinguished from *obscuriventris* by the smaller size of the female, the more shining surface of both female and worker, the deeper clypeal fossae, the more bead-like funicular joints, the ecarinate clypeus, and by the lack of pilosity on the dorsal surfaces of both female and worker. In addition *fossaceps* has a somewhat differently shaped head.

From *rufa integra,* an eastern North American form with very sparse pilosity, *fossaceps* can be differentiated by the much deeper clypeal fossae, the proportions of the funiculi, and the shining integument. From *rufa mucescens,* a Colorado form with females of about the same size, *fossaceps* can be distinguished by the shining rather than opaque integument, and very sparse rather than very dense pubescence. The writer has not seen specimens of *mucescens* and thus cannot make any statements regarding the depth of the clypeal fossae. *F. fossaceps* can be separated from *rufa integroides* varieties *haemorrhoidalis* and *ravida,* two Rocky Mountain forms without pilosity, by its much less robust queen, shining integument, very sparse pubescence, and by the unusual clypeal fossae and funicular joints.

F. fossaceps is not closely allied to any species of the *microgyna* group, all of which have much smaller females, are more or less pilose,

and have the clypeal fossae more normal in depth. Although *fossaceps* resembles *dakotensis* and its varieties in the smoothness of its integument, it is not closely related to the latter species as shown by the divergent shapes of the head and petiole.

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

Lasius (Acanthomyops) pubescens, n. sp.

WORKER

Length, 3.35–4.06 mm.

Head, excluding the mandibles, as long as broad (about .88 mm.), with straight or feebly convex posterior border and moderately convex sides. Mandibles with 6 strong teeth and 1 or 2 indistinct denticuli, basal borders edentate. Front convex, the frontal furrow absent. Scapes surpassing the posterior lateral corners of the head by one-seventh of their length. Funiculi feebly incrassated, the penultimate joint slightly longer than broad; last joint broader than the penultimate, and a little longer than the ninth and tenth taken together. Eyes hairy, with about 45 facets. Maxillary palpi short, 3-jointed. Pro- and mesonotum moderately convex. Meso-epinotal depression shallow. Epinotum without distinct base or declivity, evenly rounded. Petiole small, narrow, with a transverse, truncate superior border which may be slightly excised but not notched, blunter than in *L. (A.) interjectus* and *L. (A.) claviger.*

Head, thorax, and gaster strongly shining except where somewhat obscured by the pubescence.

Erect hairs short and sparse, shorter and less flexuous than on *interjectus,* lacking, however, only on the scapes, tibiae, and middle and hind femora. Hairs scattered over the surface of the gaster as in *claviger;* more sparse than in *claviger,* however. Pubescence very dense for *Acanthomyops.* Pubescence a little more abundant on the head than on *interjectus;* sparser on the thorax than on the head, but, nevertheless, much more dense than on *interjectus;* dense on the gaster, somewhat concealing the surface.

Color varying from light to brownish yellow, the head never reddish.

FEMALE

Length, 4.79–5.18 mm.

Head, excluding the mandibles, a little longer than broad, with feebly excised posterior border and slightly convex sides. Mandibles 6-toothed, basal borders edentulous. Clypeus weakly subangulate in front. Frontal carinae short, scarcely distinct. Eyes hairy, small but quite convex, situated a little farther to the sides than in *interjectus.* Scapes thickened distally, surpassing the posterior lateral corners of the head by one-seventh of their length. Funiculi moderately incrassated, joints 2–9 as broad as or only slightly broader than long, the penultimate joint about as long as broad, the second joint slightly over one-half as broad as the penultimate. Maxillary palpi short, 3-jointed. Thorax distinctly less broad than the head; with about the same shape as *inter-*

jectus; the mesothorax a little less flattened dorsally. Petiole small, in profile triangular, with nearly straight anterior and posterior surfaces, the superior border blunt; seen from behind, narrow, the superior border imperceptibly notched and truncate. Femora and tibiae not flattened. Wings about 5.5 mm. long.

Thorax shining, head less so; gaster subopaque, the surface obscured by the dense pubescence.

Pilosity of about the same arrangement as in *interjectus,* but much sparser, much shorter and less flexuous. Hairs very few on gula, front, occiput, and petiole, none on the vertex. A few hairs unevenly distributed along the posterior borders of the gastric segments, a few also scattered over the surface of the gaster. Pubescence particularly dense on the gaster, less dense on the head, sparser on the dorsum of the thorax.

Color dark grayish brown, the appendages lighter.

Described from 4 females and 113 workers taken from a sandy, low mound nest in open woodlands near Jenkins, Minn., August 11, 1941. Since no males and but few females could be found, it is probable that most of the sexual forms had already left the nest.

L. (A.) pubescens belongs with the *interjectus* group of species as shown by the long scapes and feebly incrassated funiculi. However, it does not seem closely related to the typical *interjectus,* the queens differing considerably in size, color, pubescence, and pilosity. It may be more closely related to *interjectus mexicanus* which has a queen of equal size. Judging from Wheeler's description of the latter, the queen of *pubescens* can be distinguished definitely from that of *mexicanus* by its different color, shorter wings, and probably also by denser pubescence. The worker of *pubescens* can be distinguished from that of *mexicanus* by the dense pubescence and sparse pilosity. Both workers and females can be separated from those of *interjectus coloradensis* by the sparse rather than numerous erect hairs and dense rather than sparse pubescence. From the other species with very small females, *L. (A.) occidentalis* and *plumopilosus,* it differs in characters too numerous for consideration. *L. (A.) pubescens* can be distinguished from *L. (A.) parvula* M. R. Smith, a small species from Illinois, by its sparse, short pilosity, larger eyes, and dense pubescence.

This species may be parasitic on one of the varieties of *Lasius niger.*

Lasius (Acanthomyops) clavigeroides, n. sp.

WORKER

Length, 3.48–4.02 mm.

Head, excluding the mandibles, as broad as long, with straight or slightly convex posterior border and moderately convex sides. Mandibles 8-toothed, the basal-most tooth pointing posteriorly. Clypeus rounded or feebly subangulate in front. Frontal carinae scarcely diverging behind. Scapes incrassated distally, reaching the posterior lateral corners of the head. Funiculi incrassated, the penultimate joints a little broader than long. Eyes small, hairy, with about 30–35 facets. Maxillary palpi short,

3-jointed. Pro- and mesonotum moderately convex in profile, less so than in *claviger*. Base of epinotum in profile moderately convex, declivity straight; the base about three-fifths as long as the declivity. Petiole smaller and with a blunter superior border than *claviger*, in profile with imperceptibly convex anterior and posterior faces; when seen from behind, the petiole straight or faintly excised above but not notched as in *claviger*.

Entire body strongly shining but not quite as shining as *claviger*, the surface often concealed a little by the pubescence.

Erect hairs sparser, much shorter, and less flexuous than on *claviger*, nearly absent on gula, front, and femora; more abundant on the gaster than on other body surfaces. Pubescence moderately dense on the head, sparse on the thorax, varying from rather sparse to moderately dense on the gaster. Pubescence on all regions usually more profuse than on the corresponding regions of *claviger*.

Color varying from light to brownish yellow, the head never reddish.

FEMALE

Length, 5.67–6.23 mm.

Head, excluding the mandibles, insignificantly broader than long, with feebly excised posterior border and slightly convex sides. Mandibles 7-toothed, basal borders edentate. Clypeus subangularly produced in front. Eyes hairy, moderately convex. Scapes thickened distally, not quite reaching the posterior lateral corners of the head. Funiculi clavate, the penultimate joints about 1.4 times as broad as long; the second joint a little less than one-half as broad as the penultimate. Maxillary palpi short, 3-jointed. Thorax with the shape of *claviger*. Petiole small, in profile with flat anterior and posterior surfaces, the superior border blunt; seen from behind, narrow, the sides straight above, the superior border feebly convex, sinuate, or faintly notched. Femora and tibiae slightly flattened, the fore femora about three times, the middle and hind femora about four times, as long as broad. Wings about 6.3 mm. long.

Entire insect shining, but not glabrous like *claviger*, the shining surface somewhat hidden by the pubescence.

Erect hairs sparser, much shorter and less flexuous than on *claviger;* lacking the microscopic plumosity which characterizes the hairs of many *claviger* queens. No hairs on the front, very few or none on the vertex and occiput. Pubescence shorter and much denser than that of *claviger*, particularly on the head and gaster where it is dense enough to give a slight pruinose effect; sparser on the dorsum of the thorax.

Color dark blackish brown, appendages lighter. Veins and stigma of the wings pale brown.

MALE

Length, 3.93–4.71 mm.

Head, excluding the mandibles, a little broader than long, with moderately convex posterior border and sides, a little broader behind

than in front. Each mandible with only a strong apical tooth as in *claviger*. Scapes slightly surpassing the posterior lateral corners of the head. Eyes very convex, hairy, situated in the middle of the sides of the head. Maxillary palpi short, 3-jointed. Thorax slightly narrower than the head, not as robust in profile as *claviger*. Petiole small, the superior border not very sharp; in profile, with feebly concave anterior and feebly convex posterior faces. Wings about 4.6 mm. long.

Head and thorax weakly shining, gaster moderately so.

Erect hairs shorter and sparser than on *claviger*. Pubescence rather sparse, but denser than on *claviger*.

Color black; legs and funiculi lighter.

Described from 65 virgin females, 27 males, and 110 workers taken from a sandy, low mound nest in oak woods near Solon Springs, Wis., August 15, 1941.

L. (A.) clavigeroides seems to be very closely related to *L. (A.) claviger*. In the field the worker, female, and male appear like small replicas of the corresponding phases of *claviger*. The striking differences in pilosity and pubescence entitle *clavigeroides* to full specific rank, however. The female of *clavigeroides* can be distinguished from that of *claviger* by the very short, sparse pilosity, much denser pubescence, smaller petiole which is blunter above and only minutely notched if at all, less flattened legs, and smaller size. The worker of *clavigeroides* can be separated from that of *claviger* by its smaller size, much sparser, shorter pilosity, somewhat denser pubescence, blunter, unnotched petiole, and less convex sides of the head. *L. (A.) clavigeroides* differs from *claviger subglaber* by most of the above points. In addition the queens differ considerably in color.

L. (A.) clavigeroides workers can be distinguished from *L. (A.) parvula* by their larger size, shorter, sparser pilosity and somewhat shorter, more incrassated antennae. Queens and workers of *clavigeroides* and *pubescens* can be readily distinguished by their antennae.

L. (A.) occidentalis differs from *clavigeroides* by having the petiole very large, its superior border sharp and emarginate, and by having the head of the female long, rectangular, and narrower than the thorax. The pile is also more abundant than on *clavigeroides*.

This species is probably a temporary social parasite of one of the varieties of *Lasius niger*. However, *L. brevicornis* was also present in the type locality and may possibly act as the host.

EIMERIA NEOLEPORIS N. SP., OCCURRING NATURALLY IN THE COTTONTAIL AND TRANSMISSIBLE TO THE TAME RABBIT[1]

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During the period from August through December, 1941, microscopic examinations were made of the colic content or feces of 15 cottontails (*Sylvilagus floridanus mearnsii* Allen) taken in the vicinity of Ames, Iowa. Four of the animals were found to harbor oöcysts of a hitherto undescribed species of Eimeria. A careful morphological study was made of the fresh, sporulating, and sporulated oöcysts, and the time required for sporulation in a layer of 3 per cent potassium dichromate solution of about 2 mm. depth in Petri dishes at room temperature was observed. Successful attempts were made to infect previously uninfected tame rabbits (*Lepus cuniculus*), so that it was possible to determine the prepatent periods and patent periods for infections in the latter host. The critical characters of the oöcysts have been determined as follows:

Eimeria neoleporis, n. sp.

Shape: subcylindrical or elongate ellipsoidal, usually tapering somewhat toward the micropyle.

Color: pinkish yellow.

Micropyle: present, very distinct (except in certain perhaps abnormal specimens appearing at the end of heavy infections).

Oöcyst wall: smooth, same thickness throughout, enlarging noticeably near the micropyle.

Extra-residual body: consisting of 4 or less granules at the sporoblast stage, or absent, usually disappearing after completion of sporulation.

Intra-residual body: present, large, occupying about ⅓ of the sporoblast.

Protoplasmic contents: a compact sphere in the center of the oöcyst. Toward the end of heavy infections usually many oöcysts are eliminated with the contents diffused throughout. Such oöcysts fail to sporulate.

Sporulation time: 50 to 75 hours; average 60 hours.

Length: 32.8–44.3 μ, mean, 38..8 μ; most frequent, 38.5 μ.

Breadth: 15.7–22.8 μ, mean, 19.8 μ; most frequent, 20.0 μ.

Mean shape index: 1.95.

Spores: with Stieda body, elliptical, measuring on the average 17.1 μ in length by 8.0 to 9.0 μ in width.

Sporozoites: banana-shaped as usual, with nucleus, large and small refractive granules.

[1] Journal paper No. J-976 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 570. The Fish and Wildlife Service (U. S. Dept. of the Interior), Iowa State Conservation Commission, and American Wildlife Institute cooperating.

[2] Grateful acknowledgment is made of technical assistance by Dr. E. R. Becker.

Polar inclusion of the oöcyst: not observed.
Prepatent period: 11 to 14 days; mean, 12 days.
Patent period: 8 to 16 days; mean, 10 days.
Specific diagnosis: closest to *E. leporis* Nieschulz, 1923,[3] from the intestine of the Alpine hare (*Lepus timidus*) in Europe.
Differs principally in: (1) presence of a very distinct micropyle, (2)

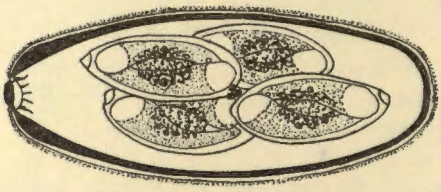

Fig. 1. Sporulated Oöcyst of *Eimeria neoleporis*. x1350.

extra-residual body (when present) of only a few granules, and (3) infectivity to tame rabbit (*Lepus cuniculus*). To date the author has obtained 5 consecutive passages through the latter host and infected in all 26 tame rabbits.

[3] Nieschulz, O. 1923. Uber Hasenkokzidien (*Eimeria leporis* n.sp.). Dtsch. tierärztl. Wochenschr., 31:245–247.

AUTHOR INDEX

Allen, Edward S., 161
Anderson, Richard L., 3
Apperson, Lester D., 7
Arnold, C. R., 519
Autrey, Kenneth Maxwell, 10

Banzon, Julian R., 15
Bates, Herbert Templeton, 19
Beam, Robert Edwin, 21
Becker, B. M., 227
Becker, E. R., 443
Benson, Richard Ernest, 28
Bindschadler, Ernest, 33
Buren, William F., 399

Caldwell, Carlyle G., 37
Campbell, F. E., 207
Carvalho, José Candido M., 409
Chi, Hsiu-Hui, 189
Clare, Stewart, 349
Clark, T. F., 451
Cochran, W. G., 421
Cook, Roy Herbert, 41

Dale, Lois W., 391
DeBord, George G., 471
Diehl, Harvey, 161
Dinger, Jacob E., 44
Drake, Carl J., 329

Eddy, Gaines W., 539
Edgar, Rachel, 391, 411
Erwin, A. T., 481

Farrankop, Helen Elizabeth, 46
Fryer, Holly Clair, 49

Gaessler, W. G., 291
Garrison, E. R., 363
Gastrock, E. A., 451
Glymph, Eakin M., 52
Goering, Kenneth Justin, 55
Griffiths, James T., Jr., 57

Haber, E. S., 291
Hager, Anna, 60
Hambleton, E. J., 329
Hammer, B. W., 363
Harris, H. M., 357
Hickey, Richard James, 63
Hicks, Ellis A., 299
Hixon, R. M., 291
Hixson, Ephriam, 66
Ho, Florence Pen, 69
Ho, Wen-chun, 72
Hoecker, Wesley Henry, 75
Holl, D. L., 319
Hsiao, Tsai Yu, 241

Jacobs, P. Burke, 227
Jensen, Christian, 77
Johnson, Ruth L., 411
Johnston, Joseph Rex, 80
Jones, Burdette, 207
Jones, S. E., 82
Joyce, Chas. R., 539
Judy, John Nelson, 85

Levine, Max, 519
Lichte, Minnie E., 411
Lindegren, Carl C., 271, 307
Lo, Shih-Ching, 88
Low, Jessop Budge, 90

McClure, H. Elliott, 93
McElhinney, T. R., 227

Manis, Hubert Clyde, 96
Moore, Francis B., 99
Moore, Frank J., 103
Moore, Fred Warren, 106

Newton, J. Marshall, 108
Nicolaysen, Howard Frederic, 111
Noakes, Frank, 114

Pacevitz, Henry Alexander, 117
Pitcher, Walter H., 120
Polderboer, Emmett B., 331
Porges, N., 451

Rayman, Morton M., 122
Reeder, Wendell, 125
Robertson, Fred, 173
Roudabush, Robert L., 437
Rowe, John A., 211, 487

Ryan, Alden Hoover, 128

Seiferle, Edwin J., 130
Semeniuk, G., 337
Shillinglaw, Clifford Allen, 132
Smith, Lawrence, 443
Stewart, Meredith Marvin, 134
Swanson, C. Loyal W., 137, 379
Sweeney, O. R., 207

Tauber, Oscar E., 349
Thorne, Charles J., 140
Tuthill, Leonard D., 145

Viado, Getulio B., 146

Williams, Neil Kenneth, 148
Willingham, Juddie Johnson, 152

Yablunky, Harry Louis, 155
Young, Charlotte Marie, 158

SUBJECT INDEX

Absorption of radiant energy in plants, 44

Acanthomyops, *see* Lasius

Acetobacter, gluconic acid production by, 451

Acetobacter suboxydans, action of, upon i-inositol, properties of compounds produced by, 120

Acetobacter suboxydans fermentation products, several, polarographic method in the investigation of, 52

Acid, gluconic, production by Acetobacter in the absence of a neutralizing agent, 451

Acid hydrolyzate of oat hulls, production of chemicals by the fermentation of, 122

Acid, pantothenic, effect of *Eimeria nieschulzi* growth-promoting potency of feeding stuffs, 443

Acids and carbon dioxide, effect of, on survival of micro-organisms, 132

Acids, mineral, as saccharifying agents for production of fermentable sugars from starch, 55

Activated carbon from certain agricultural wastes, 227

Aedes, 217
aurifer, 219
campestris, 218
canadensis, 218
cinereus, 221
dorsalis, 218
fitchi, 217
flavescens, 218
impiger, 219
nigromaculis, 217
riparius, 218
spenceri, 219
sticticus, 220
stimulans, 218
triseriatus, 220
trivittatus, 219
vexans, 220

Agricultural wastes, activated carbon from, 227

Alcohols, stereoisomeric, the number of, 161

Alkaline washing compounds, germicidal properties of, 519

Aluminum-chloride method for quantitative estimation of wool, 391

Amblyomma
americanus, 542
ovale, 542

American coot, parasites of, in central Iowa, 437

American veratrum, a chemical investigation of, 130

Amylase, mold, as a saccharifying agent for production of fermentable sugars from starch, 55

Amylase, soybean, concentration, characterization, and properties of, 108

Anhyssus tricostatus, 361

Anopheles, 212
maculipennis, 212
punctipennis, 212
quadrimaculatus, 213
walkeri, 214

Ants, new, from Minnesota, Iowa, and Wisconsin, 399

Approximations of functions by the use of functionals, errors in, 41

Archimedean screw, modified, chemical engineering applications of, 19

Argas persicus, 539

Artichokes, levulose from, 291

Bacteria, fluorescent, in dairy products, 363

Bacterial action, distribution of salt in butter and its effect on, 75

Basal metabolism, factors contributing to variability in, 158

Bionomics and morphology of the black onion fly, *Tritoxa flexa* (Wied.) (Diptera, Ortalidae), 96

Black onion fly, bionomics and morphology of, 96

Blattella germanica L., circulation of hemolymph in the wings of, 349

Blood of pregnant rats fed a diet containing dried autoclaved pork muscle, concentration of sugar in, 69

Blue cheese, relationship of the lipolytic and proteolytic activities of various penicillia to the ripening of, 77

Butterfat, effect of soybeans and soybean oil on production and quality of, 148

Calcium chloride, effects of, on motility of the isolated crop of *Periplaneta americana*, 57

Carbon, activated, from certain agricultural wastes, 227

Carbon dioxide and acids, effect of, on survival of micro-organisms, 132

Cassava, fermentative utilization of, 15

Catalysts, copper oxide, in the hydrogenation of furfural, some metallic oxides as promoters for, 134

Chaetomium funicola Cke., dissimilation of glucose by, 337

Cheese, blue, relationship of the lipolytic and proteolytic activities of various penicillia to the ripening of, 77

Chemical engineering applications of a modified archimedean screw, 19

Chemical investigation of American veratrum, 130

Chemicals in several fermentations, effect of controlled pH upon the production of, 63

Chemicals, production of, by the fermentation of the acid hydrolyzate of oat hulls, 122

Chicory, levulose from, 291

Chilean Rhopalidae in the Edwyn C. Reed collection, 357

Chinese Miridae, a list, with keys to subfamilies, tribes, genera, and species, 241

χ^2 correction for continuity, 421

Chloral in the gaseous phase, condensation of mercaptans with, 99

Circulation of hemolymph in the wings of the cockroach, *Blattella germanica* L., 349

Cockroach, circulation of hemolymph in the wings of, 349

Colloides n. gen., 476
anoxydana n. sp. 476

Concentration, characterization, and properties of soybean amylase, 108

Concentration of sugar in blood of pregnant rats fed a diet containing dried autoclaved pork muscle, 69

Condensation of mercaptans with chloral in the gaseous phase, 99

Conjunctivitis, new species of Neisseria from, 471

Consolidation of elastic earth layers, 319

Continuity, the χ^2 correction for, 421

Continuous space frames, analysis of, by three-dimensional moment-distribution and slope-deflection methods, 88

Coot, American, parasites of, in central Iowa, 437

Copper oxide catalysts in the hydrogenation of furfural, some metallic oxides as promoters for, 134

Corn, sweet, origin of, 481

Correction, χ^2, for continuity, 421

Correlation, serial, in the analysis of time series, 3

Cotton flea hopper, host relation of, 66

Cotton prematurely killed by cotton root rot, effect of insect control on the yield and quality of, 82

Cotton root rot, effect of insect control on the yield and quality of cotton prematurely killed by, 82

Cottontail, *Eimeria neoleporis* occurring naturally in, transmissible to the tame rabbit, 409

Culex, 221
apicalis, 221
erraticus, 224
pipiens, 224
restuans, 223
salinarius, 223
tarsalis, 222

Dahlias, levulose from, 291

Dairy cattle, physiologic and economic efficiency of rations containing different amounts of grain when fed to, 10

Dairy products
action of mold inhibitors on, 152
fluorescent bacteria in, 363

Dermacentor variabilis, 542

Dielectric constant of gases at ultra-high frequencies, 128

Dietary modifications, effects of, on the tapeworm *Hymenolepis diminuta*, 60

Diet containing dried autoclaved pork muscle, concentration of sugar in blood of pregnant rats fed a, 69

Differential equations, linear, solution of, by the use of functionals, 140

Dinitrophenols, internal effects of, on insects, 146

Dissimilation of Glucose by *Chaetomium funicola* Cke., 337

Divalent organolead radicles, 8

Dropping analysis, seasonal food preference trends of eastern ruffed grouse by, 331

Earth layers, elastic, consolidation of, 319

Eastern ruffed grouse in Iowa, seasonal food preference trends, 331

Ecology and management of the mourning dove, *Zenaidura macroura* (Linn.), in southwest Iowa, 93

Ecology and management of the Redhead, *Nyroca americana* (Eyton), in Iowa, 90

Economic efficiency of rations containing different amounts of grain when fed to dairy cattle, 10

Eimeria neoleporis n. sp., occurring naturally in the cottontail and transmissible to the tame rabbit, 409

Eimeria nieschulzi, growth-promoting potency of feeding stuffs, nature of, 443

Elastic earth layers, consolidation of, 319

Electron-sharing ability of radicals and the ionizing power of non-aqueous solvents, relationship between, 103

Energy, radiant, in plants, absorption of, 44

Engineering, chemical, applications of a modified archimedean screw, 19

Equations, linear differential, solution of, by the use of functionals, 140

Feeding stuffs, nature of *Eimeria nieschulzi* growth-promoting potency of, 443

Fermentable sugars from starch, mineral acids and mold amylase as saccharifying agents for production of, 55

Fermentation of the acid hydrolyzate of oat hulls, production of chemicals by, 122

Fermentation products, several *Acetobacter suboxydans*, polarographic method in the investigation of, 52

Fermentations, several, effect of controlled pH upon the production of chemicals in, 63

Fermentative utilization of cassava, 15

Fetal livers of rats fed a diet containing dried autoclaved pork muscle, glycogen in, 46

Fixed nitrogen content of power plant flue gases, determination of, 207

Flea hopper, cotton, host relation of, 66

Fluorescence associated with proteins, 125

Fluorescent bacteria in dairy products, 363

Fly, black, onion, bionomics and morphology of, 96

Food preference trends of eastern ruffed grouse in Iowa, seasonal, 331

Formica
fossaceps, n. sp.
reflexa, n. sp.

Fox squirrel, western, inventory methods, 299

Fractionation of starch, 37

Frames, continuous space, analysis of, by three-dimensional moment distribution and slope-deflection methods, 88

Frequencies, ultra-high, dielectric constant of gases at, 128

Fulica americana, parasites of, in central Iowa, 437

Functionals, approximate solution of linear differential equations by the use of, 140

Functionals, errors in the approximations of functions by the use of, 41

Functions, errors in the approximations of, by the use of functionals, 41

Fungi in modern genetical analysis, the use of, 271

Fungi, soil-inhabiting, attacking the roots of maize, 72

Furfural, some metallic oxides as promoters for copper oxide catalysts in the hydrogenation of, 134

Gases at ultra-high frequencies, dielectric constant of, 128

Gases, power plant flue, determination of total fixed nitrogen content of, 207

Generalized operator, properties of, 173

Genetical analysis, modern, the use of fungi in, 271

Germicidal properties of sodium hydroxide and alkaline washing compounds, evaluation of, 519

Gluconic acid production by Acetobacter in the absence of a neutralizing agent, 451

Glucose, dissimilation of, by *Chaetomium* funicola Cke., 337

Glycogen in maternal and fetal livers of rats fed a diet containing dried autoclaved pork muscle, 46

Grain when fed to dairy cattle, physiologic and economic efficiency of rations containing different amounts of, 10

Grouse, eastern ruffed, seasonal food preference trends in Iowa, 331

Growth-promoting potency of feeding stuffs, *Eimeria nieschulzi*, nature of, 443

Haemaphysalis leporis-palustris, 541

Halides, organoaluminum, 7

Harmostes
marmoratus, 359
minor, 358
raphimerus, 357

Hemolymph in the wings of the cockroach, circulation of, 349

Herellea n. gen., 476
vaginicola, n. sp., 476

Histogenesis in the roots of *Holcus sorghum* L., 189

Holcus sorghum L., histogenesis in the roots of, 189

Hopper, cotton flea, host relation of, 66

Host relation of the cotton flea hopper, 66

Hulls, oat, production of chemicals by the fermentation of the acid hydrolyzate of, 122

Hydrogenation of furfural, some metallic oxides as promoters for copper oxide catalysts in, 134

Hydrogen overvoltage of solutions, effect of presence of salts on, 85

Hydrolyzate of oat hulls, acid, production of chemicals by the fermentation of, 122

Hydroxide, sodium, germicidal properties of, 519

Hymenolepis diminuta, effects of dietary modifications on, 60

i-inositol, properties of compounds produced by the action of *Acetobacter suboxydans* upon, 120

Indian reservation, Tama (Iowa), ticks collected on, 539

Insect control, effect of, on the yield and quality of cotton prematurely killed by cotton root rot, 82

Insects, some internal effects of dinitrophenols on, 146

Internal effects of dinitrophenols on insects, 146

Interreflection of light between parallel planes, 114

Inventory methods applicable to the western fox squirrel, *Sciurus niger rufiventer*, 299

Ionic exchange materials and their uses, 28

Ionizing power of non-aqueous solvents, 103

Iowa ants, new, 399

Iowa, central, parasites of the American coot in, 437

Iowa cities, ten, mosquito light trap catches from, 487

Iowa mosquitoes, preliminary report on, 211

Iowa seasonal food preference trends of eastern ruffed grouse, 331

Iowa State College women, nutritional status of, 158

Irradiation experiments, analysis of group differences arising from a poisson distribution of observations obtained from, 49

Ixodes
angustus, 540
cookei, 541
kingi, 541
scapularis, 540
sculptus, 541
texanus, 541

Lasius (Acanthomyops)
clavigeroides, n. sp.
pubescens, n. sp.

Lateral organoalkali compounds, 117

Levulose from chicory, dahlias, and artichokes, 291

Light, interreflection of, between parallel planes, 114

Light trap, mosquito, catches from ten Iowa cities, 1940, 487

Linear differential equations, solution of, by the use of functionals, 140

Liorhyssus hyalinus, 360
Lipolyticactivities of various penicillia, relationship of, to the ripening of blue cheese, 77

Maize, soil-inhabiting fungi attacking the roots of, 72
Management of the mourning dove in southwest Iowa, 93
Management of the Redhead, *Nyroca americana* (Eyton), in Iowa, 90
Mansonia, 216
perturbans, 216
Maternal livers of rats fed a diet containing dried autoclaved pork muscle, glycogen in, 46
Meat in nutrition, 46, 69
Mercaptans, condensation of, with chloral in the gaseous phase, 99
Metabolism, basal, factors contributing to variability in, 158
Metallic oxides as promoters for copper oxide catalysts in the hydrogenation of furfural, 134
Metal-metal interconversions with organolithium compounds, 106
Metals, heave, quantitative volumetric titration of, 111
Micrococcus, nuclear apparatus and sexual mechanism in, 307
Micromorphological approach to the study of soils, 80
Micro-organisms, effect of carbon dioxide and acids on survival of, 132
Microscopic method for measuring soil pore space and for characterizing soil structure, 137
Microscopic method of studying soil structure, 379
Milk production, effect of soybeans and soybean oil on, 148
Mima n. gen., 475
polymorpha, 475
polymorpha, var. *oxidans* n. sp. n. var., 475
Mimeae trib. nov., descriptions of, 471
Mimeae n. tribe, 477
Mineral acids and mold amylase as saccharifying agents for production of fermentable sugars from starch, 55
Minnesota ants, new, 399
Miridae, Chinese, 241
Mold amylase as a saccharifying agent for production of fermentable sugars from starch, 55
Mold inhibitors, action of, on dairy products, 152
Moment-distribution method, analysis of continuous space frames by, 88
Monograph of the Psyllinae and Triozinae (Psyllidae: Homoptera) in America north of Mexico, 145
Morphology of the black onion fly, 96
Mosquitoes, Iowa, preliminary report on, 211
Mosquito light trap catches from ten Iowa cities, 1940, 487
Motility of the isolated crop of *Periplaneta americana*, effects of pH and of various concentrations of sodium, potassium, and calcium chloride on, 57
Mourning dove, in southwest Iowa, ecology and management of, 93

Neisseria
fulva n. sp., 471
gigantea, n. sp., 472
Neisseria from conjunctivitis and vaginitis, new species of, 471
New ants from Minnesota, Iowa, and Wisconsin, 399
New species of Neisseria from conjunctivitis and vaginitis, 471
Niesthrea fenestratus, 362
Nitrogen, fixed, content of power plant flue gases, determination of, 207
Non-aqueous solvents, ionizing power of, 103
Nutrition, meat in, 46, 69
Nuclear apparatus and sexual mechanism in a micrococcus, 307
Nutritional status of Iowa State College women, 158
Nyroca americana (Eyton), in Iowa, ecology and management of, 90

Oat hulls, production of chemicals by the fermentation of the acid hydrolyzate of, 122
Onion fly, black, bionomics and morphology of, 96
Operator, generalized, properties of, 173
Organoalkali compounds, lateral, 117
Organoaluminum halides, 7

Organobismuth compounds, unsymmetrical, 155

Organolead compounds, unsymmetrical, 33

Organolead radicles, divalent, 8

Organolithium compounds, some metal-metal interconversions with, 106

Organometallic compounds, relative reactivities of some, 7

Origin of sweet corn, 481

Ornithodoros kelleyi, 540

Otobius megnini, 540

Oxide, copper, catalysts in the hydrogenation of furfural, some metallic oxides as promoters for, 134

Oxides, metallic, as promoters for copper oxide catalysts in the hydrogenation of furfural, 134

Overvoltage of solutions, hydrogen, effect of the presence of salts on, 85

Pantothenic acid, effect on *Eimeria nieschulzi* growth-promoting potency of feeding stuffs, 443

Parasites of the American coot in central Iowa, 437

Penicillia, relationship of the lipolytic and proteolytic activities of, to the ripening of blue cheese, 77

Periplaneta americana, effects of pH and of various concentrations of sodium, potassium, and calcium chloride on motility of the isolated crop of, 57

Peruvian Tingitidae, two new, 329

pH, effect of, upon the production of chemicals in several fermentations, 63

pH, effects of, on motility of the isolated crop of *Periplaneta americana*, 57

Physiologic and economic efficiency of rations containing different amounts of grain when fed to dairy cattle, 10

Plants, absorption of radiant energy in, 44

Poisson distribution of observations obtained from irradiation experiments, analysis of group differences arising from, 49

Polarographic method in the investigation of several *Acetobacter suboxydans* fermentation products, 52

Pore space, soil, microscopic method for measuring, 137

Pork muscle, dried autoclaved, concentration of sugar in blood of pregnant rats fed a diet containing, 69

Pork muscle, dried autoclaved, glycogen in maternal and fetal livers of rats fed a diet containing, 46

Potassium, effects of, on motility of the isolated crop of *Periplaneta americana*, 57

Potency of feeding stuffs, *Eimeria nieschulzi* growth-promoting, nature of, 443

Production of chemicals by the fermentation of the acid hydrolyzate of oat hulls, 122

Proteins, fluorescence associated with, 125

Proteolytic activities of various penicillia, relationship of, to the ripening of blue cheese, 77

Psallus seriatus, host relation of, 66

Psorophora, 216
ciliata, 216
columbiae, 217
ferox, 216
horrida, 216
signipennis, 217

Psyllinae in America north of Mexico, 145

Quantitative estimation of wool, comparison of the aluminum-chloride and sulfuric-acid methods for, 391

Quantitative volumetric titration of heavy metals, 111

Rabbit, tame, *Eimeria neoleporis* occurring in the cottontail transmissible to, 409

Radiant energy in plants, absorption of, 44

Radicals, electron-sharing ability of, 103

Radicles, divalent organolead, 8

Rations containing different amounts of grain when fed to dairy cattle, the physiologic and economic efficiency of, 10

Rats fed a diet containing dried autoclaved pork muscle, glycogen in maternal and fetal livers of, 46

Rats, pregnant, fed a diet containing dried autoclaved pork muscle, concentration of sugar in blood of, 69

Reactivities, relative, of some organometallic compounds, 7

Redhead, ecology and management of, in Iowa, 90

Rhipicephalus sanguineus, 541

Rhopalidae, Chilean, in the Edwyn D. Reed collection, 357

Root rot, cotton, effect of insect control on the yield and quality of cotton prematurely killed by, 82

Roots of *Holcus sorghum* L., histogenesis in, 189

Roots of maize, soil inhabiting fungi attacking the, 72

Ruffed grouse, eastern, seasonal food preference trends in Iowa, 331

Saccharifying agents for production of fermentable sugars from starch, mineral acids and mold amylase as, 55

Salt in butter, distribution of, and its effect on bacterial action, 75

Salts, effect of the presence of, on the hydrogen overvoltage of solutions, 85

Sciurus niger rufiventer, inventory methods, 299

Screw, modified archimedean, chemical engineering applications of, 19

Seasonal food preference trends of eastern ruffed grouse in Iowa as shown by dropping analysis, 331

Serial correlation in the analysis of time series, 3

Sexual mechanism in a micrococcus, 307

Silks, effect of sodium thiosulfate and sodium hydrogen sulfite on, 411

Slope-deflection method, analysis of continuous space frames by, 88

Sodium, effects of, on motility of the isolated crop of *Periplaneta americana*, 57

Sodium hydrogen sulfite, effect of, on some silks, 411

Sodium hydroxide, germicidal properties of, 519

Sodium thiosulfate, effect of, on some silks, 411

Soil-inhabiting fungi attacking the roots of maize, 72

Soil pore space, microscopic method for measuring, 137

Soil structure, microscopic method for characterizing, 137

Soil structure, microscopic method of studying, 379

Soils, micromorphological approach to the study of, 80

Solution of linear differential equations by the use of functionals, 140

Solutions, effect of the presence of salts on the hydrogen overvoltage of, 85

Solvents, non-aqueous, ionizing power of, 103

Soybean amylase, concentration, characterization, and properties of, 108

Soybeans and soybean oil, effect of, on milk and butterfat production and on the quality of the butterfat, 148

Space frames, continuous, analysis of, by three-dimensional moment-distribution and slope-deflection methods, 88

Squirrel, western fox, inventory methods, 299

Starch, fractionation of, 37

Starch, mineral acids and mold amylase as saccharifying agents for production of fermentable sugars from, 55

Stereoisomeric alcohols, the number of, 161

Sugar, concentration of, in blood of pregnant rats fed a diet containing dried autoclaved pork muscle, 69

Sugars from starch, fermentable, mineral acids and mold amylase as saccharifying agents for production of, 55

Sulfuric-acid method for quantitative estimation of wool, 391

Survival of micro-organisms, effect of carbon dioxide and acids on, 132

Sweet corn, origin of, 481

Tama (Iowa) Indian reservation, ticks collected on, 539

Tapeworm *Hymenolepis diminuta*, effects of dietary modifications on, 60

Tensor Analysis, theory and application of, 21

Theobaldia, 214
inornata, 214
morsitans, 215

Three-dimensional method, analysis of continuous space frames by, 88

Ticks collected on the Tama (Iowa) Indian reservation with notes on other species, 539

Time series, serial correlation in the analysis of, 3

Tingitidae, Peruvian, two new, 329
Titration, quantitative volumetric, of heavy metals, 111
Triozinae in America north of Mexico, 145
Tritota flexa (Wied.), bionomics and morphology of, 96

Ultra-high frequencies, dielectric constant of gases at, 128
Unsymmetrical organobismuth compounds, 155
Unsymmetrical organolead compounds, 33
Uranotaenia, 214
sapphirina, 214

Vaginitis, new species of Neisseria from, 471
Veratrum, American, chemical investigation of, 130
Veratrum viride, chemical investigation of, 130
Volumetric titration of heavy metals, 111

Wastes, agricultural, activated carbon from, 227
Western fox squirrel, major factors affecting the use of two inventory methods applicable to, 299
Wisconsin ants, new, 399
Women, Iowa State College, nutritional status of, 158
Wool, comparison of the aluminum-chloride and the sulfuric-acid methods for quantitative estimation of, 391

Xenogenus gracilis, 360

Yield of cotton prematurely killed by cotton root rot, effect of insect control on, 82

Zenaidura macroura (Linn.), in southwest Iowa, ecology and management of, 93