ERRATA.

Page 12, lines 16 and 17, for *one* hundred read *three* hundred and for *one thousand* read *six hundred.*

Page 17, line 2, dele first letter in the line.

Page 168, line 12, page 177, lines 13 and 14, and page 271, line 10, for *Lemna trisulca* read *Spirodela polyrhiza.*

Page 209, line 2 of foot-note, after *but* insert *represents.*

Page 256, line 7, and page 266, line 19: *snowi* n. s. has been shown to be *hieroglyphica*, ♂.

Page 257, insert as line 8 as follows: -ken to the office produced young in ten days. The

Page 272, line 13, for *P. biguttatus* read *Pompilus biguttatus.*

Page 278, Plate V., 16, after *view* insert as follows: *a*, mentum; *b*, labial rudiment; *c*, maxillary palpi; *d*, maxilla; *e*, labrum; *f*, antenna; *g*, eye; *h*, mandible.

Page 286, line 11, drop initial *the* one line.

Page 386, line 1, for *Comstocki* read *Comstock.*

Page 399, line 17, for *specimens* read *specimen.*

Page 411, line 10, for *Michaelson* read *Michaelsen.*

Page 441, line 3 from bottom, for *66* read *68.*

Page 445, line 10 from bottom, for *57* read *58.*

Page 466, line 1 from bottom, for *Cypria* read *Cypris.*

ARTICLE XII.—*On a Bacterial Disease of the Squash-bug* (*Anasa tristis* DeG.). By B. M. DUGGAR.

INTRODUCTORY.

During July, 1895, while occupied as an Assistant in the Illinois State Laboratory of Natural History, in studying some fungous diseases of the chinch-bug I used many squash-bugs (*Anasa tristis* DeG.) for experimental purposes, their larger size making them convenient for use in certain investigations for which the chinch-bug is poorly adapted. These squash-bugs were kept in a laboratory breeding-cage, and daily supplied with fresh food and a suitable amount of moisture. They were soon observed to be dying in considerable numbers, although I could detect nothing unhealthful in their surroundings.

A fresh lot of the insects was thereupon brought from the field July 23, put into a large breeding-cage, and kept as far as possible under normal conditions. A few of the bugs recently dead in the first outbreak were broken in pieces and scattered about the earth of this new habitation, or touched to the bodies of some of the healthy individuals. A much larger number of this fresh supply of insects was reserved for another small cage, which, with conditions otherwise similar, was left uncontaminated by the bodies of any of the dead or diseased insects. In three days one half of the insects in the infected cage were dead, while in the untreated cage, with so many more individuals, there were only two or three dead. The dead bugs in the infected cage presenting common characters, and a careful microscopic examination showing a well-defined bacillus uniformly abundant, this preliminary test encouraged further experimentation with this disease from a strictly bacteriological standpoint, with a view to an elucidation of both its practical and scientific features.

PRIMARY INFECTION EXPERIMENTS.

In order to refer all cultures and subsequent results to a definite and unmistakable source, a few preliminary infection experiments were begun, the results of which were recorded in detail. It seems well, therefore, to preface particulars concerning the culture and infection work reported in this paper with the following fundamental infection records:

Experiment 1.—A box breeding-cage was stocked with about two hundred squash-bugs, both nymphs and adults, squash leaves being daily supplied as food, and kept fresh by inserting the stems through the cork of a small jar containing water. From the preliminary disease-cage several dead bugs were taken August 1, the bodies torn apart, and the pieces scattered about the earth and food leaves in the new breeding-cage. In two days ten bugs were dead, and in five days a total of sixty dead was reached. At this date a few of the dead insects were again broken in pieces and scattered about the cage, while the remainder were piled in a corner. On the eighth day the mortality reached ninety, and the following day added ten more. The death rate was now much lessened, and from August 12 to August 15 only three deaths occurred. A few insects had been removed for various purposes, and on the last-named date the number of living bugs remaining was between sixty and seventy-five, while about one hundred and fifteen deaths had occurred.

Experiment 2.—As a check on the preceding, about fifty squash-bugs of the same lot were put into a much smaller breeding-cage on the same date, August 1, and kept subject to similar conditions, but without inoculation. The first death was noted August 7, but the body of the dead insect was normal. This cage was observed until August 17, with a record of but one additional death, and this not attributable to the disease.

From the record of these two experiments it will be

seen that evidence of the contagious nature of the disease was clear, and after August 17 the cage used in Experiment 1 served as a general source of infection material. The observations show that the greatest mortality was probably from three to six days after the introduction of the disease, and no dead insects were in any case found at the expiration of the first day. The proportion of deaths was undoubtedly greatest among the younger nymphs, but many adults also succumbed. Subsequent experiments likewise confirmed this conclusion, although the difference was not always very great.

Characters of the disease.—The effect on the insect and the *post-mortem* appearances were carefully noted in Experiment 1. A few hours before death the insect may be found in a sluggish condition, resting low on its ventral surface, and often apparently incapable of raising itself erect, or of crawling without a marked drag. If placed on its back, it has no power to return to the normal position. As the disease progresses the insect loses nearly all muscular activity, and a slight waving of limbs and antennæ may be the only indication of life. Squash-bugs cannot attach themselves strongly to the leaves by their limbs; and as they usually frequent the under surface of the leaf, diseased bugs are commonly found on the earth of the cage.

A few hours before death there is no marked discoloration of the body; but the insect becomes slightly darker as death approaches, owing probably to changes in the body fluids. After death the changes are rapid. The nymphs soon assume a deep purplish black hue, the body does not shrink at all, but appears tense and slightly swollen, and in the course of twenty-four hours or more it becomes a mere sack of gruel-like fluids. In this condition the walls readily collapse, and the insect may not bear lifting without breaking. In the adults the body is observed to have a rather moist appearance at the time of death, especially in the cephalic region of the

ventral surface of the abdomen. Later, the wet appearpearance is more evident throughout; but the hard chitinous crust does not shrink or collapse, and unless broken, the offensive fluids within are unnoticed. In all cases, the odor—more pronounced and putrefactive than the normal squash-bug odor—is especially characteristic. A short time after death the appendages are very readily separable at the articulations, and it is almost impossible to lift an insect by means of them.

A long series of infection experiments will be described later, and in this connection it is only necessary to note further the *post-mortem* appearances of insects free from this disease. It has been established that bacteria are normally found in the cœcal appendages of many Hemiptera,* among which are the squash-bugs. It consequently seemed of interest to ascertain if these bacteria might become truly pathogenic, or, at least, cause the peculiar *post-mortem* appearances under abnormal conditions—as of lessened food supply. Such investigation was quite superfluous in view of the check experiment above recorded, but, nevertheless, a few healthy individuals were allowed to die from gradual starvation. Under these conditions the body cavity gradually dried out, and when death finally resulted the shell was greatly contracted in the abdominal region, and slightly drawn together ventrally. This was especially true of the nymphs, and later observations were to the same effect. Lessened vitality may encourage the disease, but it has nothing to do with a "spontaneous" occurrence. Moreover, the microscopic characters and the cultivation experiments enumerated later show conclusively that the disease form is entirely distinct from the normal form.

Other nymphs were killed by immersion in the toxic bacterial infusion from a pure culture of the disease organism,—which toxic infusion will be subsequently de-

* "Bacteria Normal to Digestive Organs of Hemiptera." By S. A. Forbes. (Bull. Ill. State Lab. Nat. Hist., Vol. IV., pp. 1—7.)

scribed,—but the usual shrinking of natural death occurred. It is probable that dead bugs placed on a wet surface might absorb moisture and show an appearance somewhat similar to the disease effect, but this is only a surmise.

ISOLATION CULTURE AND GROWTH ON AGAR-AGAR.

A number of dilution cultures were made during the progress of these preliminary experiments, but the results were so uniform that only one such culture will be reported in detail. For some of the earliest dilution cultures dead insects were necessarily employed, and the body fluids were thus greatly mixed. Even with these, however, cultures were obtained which gave one form of colony in great preponderance, while a few other forms were confined to scattered colonies in the original dish. Here, also, the abundant form was identified as a bacillus. Preliminary experiments demonstrated that this form was an effective pathogenic agent for squash-bugs; and as soon as sick insects were available, every precaution was taken to secure a culture with the least practicable admixture of foreign forms.

All the necessary apparatus having been properly sterilized, a sick nymph was thoroughly washed with corrosive sublimate solution, and a fold in the soft body wall of the abdomen was caught in the forceps, and slit with the scissors in such a manner as not to disturb the alimentary organs. A small amount of the exuded body fluid was then transferred with a platinum needle, in the usual manner, to a tube containing peptonized nutrient agar-agar at the prescribed temperature. After shaking well, several transfers were made from this first tube to a second, and finally to a third; and the contents of each were poured into a Petri dish. To the nutrient agar used in these tubes was added a small quantity of squash-leaf decoction, and as this was found to be of advantage, it was continued in all subsequent agar cultures.

In four days the dish from the original tube gave abundant colony formation, and in the next dish about ten colonies appeared. The surface colonies were circular, slightly yellowish white or dirty white, but with a distinct opalescence. The submerged colonies were elliptical or slightly pyriform in vertical projection, growing, toward the surface, more and more laterally compressed. This peculiar growth of submerged facultative anaërobic bacteria has been well explained by Professor H. Marshall Ward,* who has studied in detail under high powers the formation of micro-colonies in certain cell cultures. The horizontal long diameter of such elliptical colonies is in the plane of fission of the rodlets. The form of growth is due to the fact that as the elongating rods are broken up into daughter cells, these cells slip by each other (one over the other) under the pressure of the contracting gelatine or agar; and if in agar, evidently this form will be retained in the macro-colony. To return to the circular colonies, those that had room for the fullest development showed in a few days marked lobulations, and sometimes beautiful fan-like radiations. Furthermore, those submerged colonies that had reached the surface took on the circular form and very soon developed some of the characteristic radiations, although the growth is thinner than when they develop superficially. All stages of these developments are evident in Plate XXVIII., Fig. 1. Submerged colonies appear deeper colored than the surface ones, but this is only an apparent coloration. It will also be seen that there is a film-like growth on the lower surface of the agar beneath deeply submerged colonies. This, also, is the same form which has spread out between the contracted agar surface and the glass.

The lobulated growth appearance is not always present, even in cultures direct from the insect, and like all such radiating bacterial growths, is probably greatly

* "The Formation of Bacterial Colonies" (Annals of Botany, Vol. IX., No. XXXVI., Dec., 1895.)

affected by the amount of moisture, by the temperature, and by all conditions affecting the vitality of the organism. These radiations are apparently more abundant where the amount of moisture is considerable and the temperature about the *optimum*, but with too much moisture the bacillus spreads over the surface as a continuous sheet-like growth. Where the colonies are very abundant they remain small and circular, or become variously united in a complete network. At low temperatures the lobulations seldom occur. I have also made Petri-dish cultures from tubes kept for some time in the laboratory, and on agar containing varied quantities of water, but these marked growth characters were then entirely absent. This may be due to a lessened motility of the bacillus, or to other causes not wholly understood.

After being grown in the laboratory in several successive tube cultures, this organism seems to lose the power of producing the slight yellowish-white color, and the growth becomes a purer opal white.

It should be noted that in subsequent isolation cultures, it was found that the pathogenic form could often be secured pure, even in the first dish, by using sick insects, observing proper sterilization precautions in clipping off a leg in the region of the femur, and transferring to the tube with a platinum needle a little of the exuded fluid. The fluids of the diseased bug are almost pure cultures of the pathogenic organism, and unless the alimentary tract is badly broken down, I have seldom found many foreign germs.

From the above notes it will be seen that with this bacillus there is apparently no tendency towards spontaneous variation in the colonies growing side by side in a culture direct from the insect. I have also carefully observed the growth in cultures from various sources, and the more marked opalescence after continued growth on agar is the only variation noted.

GROWTH CHARACTERS WITH VARIOUS NUTRIENT MEDIA.

Solid media.—On nutrient gelatine this organism makes a rapid growth. Several stab cultures were made on Sept. 30, and the tubes were kept at a cool, living-room temperature. In two days the central needle-path showed a growth throughout its whole extent, and on the third day liquefaction had begun. The appearance is at first that of a short cylindrical or rotund air cavity, from the lower surface of which projects the tapering liquefied portion for about one third of the inoculation line, showing a considerable precipitate in the lower part. On the third day the liquefied portion was considerably extended, occupying an area shaped like an inverted lamp chimney, along the middle line of which extended the undulating precipitate-like bacterial formation. In the course of one week, three fourths of the gelatine was liquefied, and the bacterial growth was scattered through the lower portion in a flocculent manner. The remainder of the gelatine was soon liquefied, the flocculent material settled to the bottom, and with the exception of a slight turbidity the color of the liquefied portion remained unchanged. After standing for about one month, and before any great amount of evaporation had taken place, the light amber-color was changed to a deep reddish amber, and the color became more pronounced as evaporation advanced.

On slices of sterile potato kept in a moist chamber, this organism makes a profuse growth in two days. About this growth there is nothing especially typical; but the dirty-white color is well marked, and the thick film of growth shows a strong tendency to become lobulated on the margins.

Liquid media.—Fluid cultures were made to determine the nature of the growth, and also to ascertain how the organism might be propagated in considerable quantity for infection experiments, if the latter should prove desirable. In bouillon a considerable turbidity is produced

in the course of two days; and with further growth a slight film is developed on the surface, and a precipitate begins to accumulate at the bottom. This precipitation increases with the maturity of the culture, the superficial film disappears, and the liquid is left slightly clouded and very little darker than the uninoculated fluid. After the cessation of growth, there is no further change of color in the bouillon. Other cultures were made on decoctions of green corn, of squash leaves, and also on a mixture of bouillon with each of these. The growth differed little from that on bouillon, except in a thicker, scum-like surface film.

Sterile skimmed milk inoculated from a pure culture of the squash-bug bacillus shows considerable curdling in twenty-four hours, and subsequently a rapid production of whey. In one week the curd is about half dissolved, and it soon assumes a somewhat rusty color, as also does the turbid whey. A few days after inoculation the odor from these milk growths is extremely disagreeable, and it becomes vile and penetrating. It resembles the odor of sulphureted hydrogen, and, while not so strong, it is more nauseating. An infusion of the bacillus from a growth on agar was sterilized at a low temperature, and then added to a small quantity of sterile skimmed milk. No change was produced in the milk, and a subsequent inoculation with the active bacillus gave a growth characterized as above.

From the experiments recorded under the head of "Toxic Properties" it is noted that an infusion* of this organism from an agar culture contains some substance fatal to insects, and various media were employed in order to get this substance in a form suitable for chemical analysis. Buckmaster† has mentioned some nutrient media used by Uschinsky for the cultivation of certain

* It is well to note here that in this paper I have used the word infusion only to denote the fluid in which bacteria have been diffused for inoculation or other such purposes, and no idea of heat is thereby implied.

† *Ursprung und Beschaffenheit gewisser Bakteriengifte.* (Biol. Centr. Bd. XV., Nr. 3, Feb., 1895.

pathogenic organisms, by means of which a toxic excretion of bacteria may be secured in a way convenient for chemical study. The mixture which I have tried is formulated as follows:

Water	1,000
Glycerine	40-50
Sodium Chloride	5-7
Ammonium Lactate	10
Calcium Chloride	0.1
Magnesium Sulphate	0.2
Potassium Biphosphate	1

To the above formula Uschinsky added a little sugar for some organisms, and urea or uric acid for others. With the bacillus of this squash-bug disease an addition of 5% sugar gave an abundant growth, but the development was very slow. Three weeks after the inoculation a thick yellowish white film had formed on the upper surface, and the bottom of the flask was likewise thickly covered with a sedimentary deposit. During the first two weeks' of growth the liquid had a slight pinkish coloration. An addition of 10% sugar gave much less growth than the above, but the pink coloration was more marked. Urea to the amount of one half per cent. with the formula mentioned, also urea one half per cent. and sugar 5% in each of two other flasks, differed very little from the culture first described, although the pink color was more lasting, and possibly the growth of less extent. The value of these media for securing the toxic principle for analytical purposes cannot yet be determined, as the chemical work has not been completed.

A culture consisting of fermentation broth in bent tubes gave no gas production. The ingredients of the broth used were as follows:

Water	1,000 c.c.
Glucose	20 grms.
Peptone	10 grms.
Sodium Chloride	5 grms.

In a nitrate solution the organism grows well, but there is no reduction of the nitrates, as shown by negative results (absence of red color) from the usual test—

the addition of a few drops of naphthylamine chloride and a small quantity of sodium sulphanilate. The formula for the nitrate solution used is as follows:

Water	1,000 c.c.
Peptone	1 grm.
Potassium Nitrate	0.2 grm.

INFECTION EXPERIMENTS WITH THE SQUASH-BUG.

Laboratory Experiments.

In addition to the introductory experiments it is necessary to describe in some detail the numerous infections made in the laboratory under various conditions. It was my practice to accompany every experiment, or series of experiments, with a check subjected to similiar conditions, but without inoculation. Moreover, with all the cages and contents of cages, sterilization by means of heat and corrosive sublimate was employed as far as was compatible with the size and nature of the materials employed. Whenever possible, the bugs used for experimental purposes were first kept in the laboratory for two or three days, in order to watch for any "spontaneous" outbreak of the disease.

Experiment 3.—In a breeding-cage which had served as a check on some previous work, there were twenty-eight adult squash-bugs and twelve large nymphs. These insects had remained perfectly healthy throughout, and on August 12 each of the individuals was inoculated by touching to its body the mixed fluids from a diseased bug out of Experiment 1. In four days eighteen bugs were dead, ten adults and eight nymphs; on the fifth day twenty-four were dead, fourteen being adults and ten nymphs; and during the next four days there were only six deaths, one nymph and nine adults remaining. A second inoculation was then made in the same manner as before, and five days thereafter one half of the remaining ten were dead. Four adults survived both inoculations. In this experiment it was noticeable that the nymphs died rapidly during the first few days.

Experiment 4.—One of the most successful experiments with the organism of this disease was the result of testting the bacillus found so abundantly and almost pure in one of the early isolation cultures. A young colony direct from a Petri dish was diffused in a small quantity of water, and each of five squash-bugs was thoroughly wet with the infusion. Three of the insects were dead on the morning of the second day, and at the end of two and one half days all were dead. A check lot of bugs used in conjunction with this experiment remained healthy.

Experiment 5.—About forty squash-bugs were inoculated from diseased insects, the cage being without moisture except that furnished by the food leaves. The results indicate that the disease took rapid effect, as fifteen bugs were dead at the end of five days. From this time the death rate diminished, but the bugs dropped off gradually until only two or three apparently resistant ones remained.

In order to compare the effect of pure cultures of various ages with infusions direct from diseased insects as sources of infection, a series of experiments were made in each of which six bugs were used. When pure cultures were employed, some of the bacterial growth was diffused in distilled water, and in this the insects were momentarily immersed. With the diseased or dead bugs an infusion was likewise prepared by tearing the bodies apart in water. Healthy bugs from two different fields furnished the subjects, and a separate check was used for each lot. All dead insects were removed as soon as observed, in order that the results might follow only from the first infection.

TABLE I.

Experiment No.	No. Insects	Source of Infection.	Number of Deaths.					Number of bugs remaining.
			2 days.	3 days.	6 days.	10 days.	Total.	
6	6	Insects from Experiment 1..	3	(1	escap	ed)	3	2
7	6	Isolation culture one month old.....	.....	1	.....	.....	1	5
8	6	Bacillus from pure culture three weeks old.....	1*	.....	2	.....	3	3
9	6	Bacillus from pure culture one week old.....	.....	.....	2	1	3	3
10	6	Bacillus from pure culture one day old.....	2	2	(1 esc	aped)	4	1
11	6	Dead chinch-bugs from previous experiment.....	1	(1	escap	ed)	1	4
12	5	Dead grasshopper previously exposed to disease.....	.....	.....	.....	.....	.....	5
13	6	Uninoculated: Check on Nos. 6, 9, 10, 11, and 12.....	.....	(2	escap	ed)	.....	4
14	6	Uninoculated: Check on Nos. 7 and 8.....	.....	.....	.....	.....	.....	6

These experiments further demonstrate the effectiveness of the isolated bacillus, and they also indicate that fresh cultures are more active than old ones. The tube used in Experiment 10 was inoculated the previous day from the tube subsequently used in Experiment 9. This series would also indicate that fresh cultures are as effective for infection as infusions direct from diseased insects. From general observations, however, I should judge that the most effective infection material is from insects recently dead. Experiment 12 is difficult to explain, since a culture from this same grasshopper seemed

* This insect was probably a spent individual of the first brood, and not diseased, as the *post-mortem* characters were not at all indicative of disease.

to yield the disease organism; and, moreover, one of the bugs immersed for a very short time in the infusion from this grasshopper was killed. This grasshopper had been dead for about two days, however, and this may have injuriously affected the disease organism.

Field Experiments.

Experiment 15.—A careful examination of a squash patch on the Agricultural Experiment Station grounds on Sept. 17 resulted in a find of two dead squash-bugs; but microscopic examination showed that the bacteria in the bodies of these insects were different from the disease bacillus. It was doubtful if the disease existed in the field at all at this time, and a field infection test was greatly to be desired. For this purpose several dead bugs from Experiment 1 were teased out in about 40 cc. of water, and this infusion was sprayed upon a squash-vine containing about two hundred bugs. This vine was somewhat isolated from the remainder of the plat. No attempt was made to get the infusion on all of the bugs; but the spray was simply directed to those leaves containing the largest number of insects. The result of the first infection alone was desired, so the dead bugs were removed as soon as found. Three dead insects and three distinctly sick, all nymphs, were removed on the second day, the true disease bacillus being found in all of their bodies. On the third day eight dead insects were removed, and the number of bugs on the vine was much reduced, owing to migration for fresher food supply. One week after infection the total number of diseased insects amounted to eighteen; but before this time nearly all of the bugs had migrated, and the experiment was discontinued.

Experiment 16.—Although the season was far advanced, and the weather probably too cool for the best results, I was encouraged to repeat Experiment 15, with precautions against migration. An infusion of ten dead

bugs out of previous experimental lots was employed in the same manner as above, in this case one gill of water being used. This was sprayed upon two hundred or more half-grown nymphs and adults infesting a squash-vine, and the vine then securely covered with mosquito netting. The weather continued quite cool, and on the fourth day after infection fourteen dead bugs were found, all but two of which showed the disease characteristics. On the seventh day there was a total of thirty-two dead bugs. A frost the previous day killed the food leaves, but the stems were still in good condition. This cold so chilled the bugs that they moved very little during the early part of the day; yet an examination of the field, as a check, gave no dead insects which might not have died from injuries by persons passing about. On the tenth day thirteen recent deaths had resulted; and in spite of the precautions taken, many of the bugs had escaped, the wind having frequently displaced the netting. After the twelfth day, October 9, the netting was removed and only one additional count made, the small number of bugs remaining not justifying further observations. A summary of the results is as follows:

September 27, about two hundred squash-bugs were sprayed with infusion of diseased bugs. October 1, fourteen dead insects were counted, and later, additional ones, as follows: October 4, eighteen; October 7, thirteen; October 9, seven; and October 15, three—a total of fifty-five.

Experiment 17.—Parallel with the above and on the same date (September 27), an experiment was made to test in the field the efficiency of old pure cultures of this organism. The growth from two tubes one month old was diffused in a gill of water, and sprayed upon the insects as before. There were, however, more than two hundred bugs present under the netting in this experiment. Four were dead October 1, and subsequent counts of additional dead were as follows: Oct. 4, two; Oct. 7,

thirteen; Oct. 9, four; and Oct. 15, one—making a total of twenty-four.

As the weather was unfavorable for the spread of the disease when once established, these experiments must be judged wholly in the light of results accruing from the original infection alone. Evidently the old cultures were not as effective as the infusions from dead bugs, and the difference is even greater than is apparent from the summaries, for there were more insects in Experiment 17 than in No. 16, and fewer bugs escaped towards the later periods of observation. There is every reason to believe that this disease may be spread among squash-bugs in the field.

INFECTION EXPERIMENTS WITH THE CHINCH-BUG (*Blissus leucopterus Say*).

The first infection experiments with chinch-bugs, made in the early part of August, were wholly unsatisfactory, owing to the spent condition of the bugs of the first brood, and the results are not included in this paper. When the second brood began to make its appearance, late in August, all previous experiments were repeated and new ones were begun.

The majority of bugs involved in the series of experiments tabulated below were in the first stage—a few in the second. In each case a large tumbler about one third filled with moist sand and covered with a muslin cloth was the cage extemporized as best adapted for this purpose. The vessels and the sand were previously sterilized, and stalks of Indian corn cut into suitable pieces were regularly supplied as fresh food. A small pill-box of the bugs, approximately five hundred, were then momentarily immersed in the infusions used, or immediately put into the cage with the infection material. The number of dead bugs in each cage was ascertained by actual count, and is given below.

TABLE II.

Experiment No.	Source of Infection.	Number of Deaths.					Number of bugs remaining.
		3 days.	5 days.	7 days.	10 days.	Total.	
18	Pure culture from first isolation—10 days old	76	23	16	5	120	Many.
19	Pure culture from second isolation—5 days old	210	57	64	29	360	About 50.
20	Check-bugs momentarily immersed in distilled water	24	1	2	4	31	Very many
21	Pieces of diseased squash-bugs from Experiment 1	71	152	155	106	484	About 100.
22	Infusion of diseased squash-bugs from Experiment 1	163	89	59	25	336	About 25.
23	Check, untreated	20	3	1	9	33	Very many
24	Additional check, untreated	15	3	4	6	28	Very many

A few dead bugs were left in the cage each time in order to insure continuous infection if the disease were established, and the forceps used in removing the insects were sterilized before passing from one cage to another. In considering the figures in the above table, however, the number of bugs remaining in each experiment at the close must enter into our estimates, for it was imposible to do more than roughly measure the insects when introduced. The results indicate that under the conditions prevailing this disease may be readily communicated to young chinch-bugs, whether the organism is used direct from diseased insects or from pure cultures. Microscopic examination also showed that the usual disease bacteria were present in the bodies of dead insects. In the case of chinch-bugs, the *post-mortem* appearances are not so characteristic as those cited for squash-bugs. The bodies are often slightly swollen, and

they do not dry out as rapidly as those dying naturally. Moreover, the color is apparently somewhat dulled.

The success of the experiments in Table II. led to the institution of a series of tests dealing with the effectiveness of chinch-bugs dead from the disease as a source of infection. The number of bugs, style of cage, and other conditions were similar to those in the preceding series. Many of the bugs had now passed the second molt. The following table summarizes the results.

TABLE III.

Experiment No.	Source of Infection.	Number of Deaths.					Number of bugs remaining.
		3 days.	5 days.	7 days.	10 days.	Total.	
25	Dead bugs from Experiments 21 and 22	35	76	24	25	160	Few.
26	Infusion of dead bugs from Experiments 21 and 22	206	79	26	28	339	Few.
27	Dead bugs from check Experiment No. 20	10	8	3	3	24	Many.
28	Check—untreated	8	6	1	3	18	Many.
29	Check-bugs momentarily immersed in water	12	7	2	7	28	Many.

Experiment No. 26 showed a greater number of deaths than No. 25, but the number of bugs remaining at the close was about equal in each, the former having contained at the beginning more bugs than the latter. The series is almost as conclusive as Table II. in showing how effectively the disease may act upon young chinch-bugs; and it further supplements the results of the latter series in showing that the dead bugs from those infected cages were capable of producing the disease anew, while dead bugs from the check had no such power.

The results with young chinch-bugs were so encouraging that a preliminary infection-box experiment was immediately begun. Squash-bugs recently killed by the

disease were broken in pieces and thrown about in the box, and numbers of young chinch-bugs were introduced. In a few days some dead insects were found, but after a time the disease seemed to die out. Most of the insects were reaching the pupa state, and experiments were discontinued until the bugs reached the adult condition.

When the adult bugs became abundant, Mr. W. G. Johnson, an Entomological Assistant in the Laboratory, conducted several infection-box experiments with various diseases, and in one of these the bacillus of the squash-bug disease was used. Every effort was made to get the bugs infected, and they were thoroughly wet with the spray of infusions rich with the bacillus direct from fresh pure cultures. Very few bugs died in this box; indeed, no more than died in other boxes with other diseases, and apparently no more than in the check box. The failure of these experiments with adult chinch-bugs, and on a scale so much larger than the tumbler experiments which I had previously concluded, somewhat chilled the prospect of pushing field experiments.

It was now necessary to test the organism on adult chinch-bugs under the conditions prevailing in the experiments given in Tables II. and III., in order to compare by actual count the death rate of the old bugs with that of the young. In this instance two tumblers were employed for each experiment. The sand in both jars was very slightly moist at the beginning; but in one it was allowed to dry out gradually, while in the other it was kept moist. As before, about five hundred bugs were used in each tumbler. The bugs were brought from the field in quantity, and as some appeared to be stifled, all dead bugs were removed from each of the cages after twenty-four hours, in order to avoid any error from outside sources. When infusions are mentioned, temporary immersion of the insects to secure infection must be understood. The following table gives all essential data.

TABLE IV.

Experiment No.	Source of Infection.	Condition.	Number of Deaths.		
			4 days.	10 days.	Total.
30 *a*	Dead squash-bugs from Experiment 1	Dry.	30	100	130
30 *b*		Moist.	42	144	186
31 *a*	Infusion from isolation culture three weeks old	Dry.	18	22	40
31 *b*		Moist.	11	19	30
32 *a*	Infusion from pure culture two weeks old	Dry.	15	20	35
32 *b*		Moist.	12	27	39
33 *a*	Infusion from pure culture one day old	Dry.	19	46	65
33 *b*		Moist.	19	38	57
34 *a*	Dead chinch-bugs supposed to be diseased	Dry.	22	39	61
34 *b*		Moist	18	30	48
35 *a*	Check—untreated	Dry.	5	24	29
35 *b*		Moist.	10	19	29

With the differing conditions and the various sources of infection employed, it would seem that there is no chance for misinterpreting the above results. The effect upon young chinch-bugs, as seen in Tables II. and III., was rapid and marked, while all experiments upon adults were slight in effect, and apparently less effective in proportion as the bugs were unconfined and more nearly free to exercise the liberty of the field. A field test was also attempted, but no beneficial results could be noted, and final experimentation along this line was necessarily deferred.

INFECTION EXPERIMENTS WITH THE BOX-ELDER BUG (*Leptocoris trivittatus* Say).

During a cold season in the early part of October, many box-elder bugs were found under a tree near the

laboratory, the cold weather having caused them to fall to the ground. Their food supply was then very scant, as the leaves were drying rapidly; nevertheless, the bugs were used for experimental purposes, as it was desirable to test the squash-bug disease on as many Hemiptera as possible. Small box breeding-cages were used, but it soon became impossible to find suitable food. *Post-mortem* appearances and microscopic examination guided my opinions as to the presence of the disease, and the series was early abandoned on account of the condition of the bugs. The appended table is not, however, without interest.

TABLE V.

Experiment No.	No. bugs.	Source of Infection.	Second Day.		Sixth Day.		Total.	
			Dead.	Diseased.	Dead.	Diseased.	Dead.	Diseased.
36	12	Infusion from diseased squash-bugs.....	2	2	3	2	5	4
37	12	Infusion of the bacillus from isolation culture	1	1	Escaped.		.....	.....
38	12	Infusion of the bacillus from old pure culture.	.....	.....	5	5	5	5
39	12	Infusion of the bacillus from fresh pure culture	1	1	5	3	6	4
40	12	Check—untreated	1	.....	2	.....	3	.....

INFECTION EXPERIMENTS WITH GRASSHOPPERS.

During the progress of this work with the squash-bug bacillus, grasshoppers dead with a supposed contagious disease were received from Prof. C. P. Gillette, Entomologist of the Colorado Agricultural Experiment Station. For a time the two diseases were not clearly distinguished, and some parallel experiments were conducted. It is only necessary here to mention some attempts to inoculate grasshoppers with the squash-bug bacillus. In one

cage four grasshoppers were brushed externally with the body fluid from diseased squash-bugs, and in another cage the infection material was from a pure culture of the bacillus ten days old. In the course of ten days two hoppers were dead in each cage, as also in the check lot. With the hoppers from the inoculated cages, isolation cultures, growth on gelatine, and microscopic characters indicated that the bacillus was that of the squash-bug disease; but the only attempt made to inoculate squash-bugs from these dead grasshoppers gave very slight result.

INFECTION EXPERIMENTS WITH GRUBS AND CATERPILLARS.

No true bacterial disease of Hemiptera has previously been reported, and, with few exceptions, these diseases have been confined, as far as known, to lepidopterous and coleopterous insects. In this connection it is to be remembered that *Bacillus insectorum* (*Micrococcus insectorum* Burrill) of the chinch-bug was finally located as a "normal" form,—one of various normal forms common in the cœcal appendages of the higher Hemiptera,—and it consequently cannot be termed pathogenic with our present knowledge of the subject. These facts make experiments with the squash-bug bacillus on grubs of beetles and on caterpillars greatly to be desired.

White grubs, probably larvæ of *Lachnosterna fusca*, were inoculated externally both with fluids from diseased insects and with fresh pure cultures; but in no case was there any successful infection. Four grubs were used in each experiment, and they were kept under observation for about three weeks.

Fall web-worms were also exposed to this disease by touching to their bodies the fluids of diseased squash-bugs, smearing the material over the food leaves, and scattering bits of the diseased bugs about the cage; but the disease took no effect. A few other preliminary experiments were attempted, which are briefly summarized below.

A tomato-worm (Protoparce) nearly full grown was smeared along the line of spiracles with the diseased fluids. Suitable food could not be obtained, but the larva lived ten days, and gradually shrank in size and partly pupated. At this time a microscopic examination was made, but no bacteria could be found in the tissues or in the fluids.

Following the above, a larva of the white-lined morning sphinx, *Deilephila lineata* Fab., was inoculated by clipping off its horn and injecting into the body a small quantity of an infusion from a pure culture. The larva died in two days, filled with bacteria of several kinds, and four squash-bugs were then inoculated from this larva. At the end of two days one of these bugs was dead, but the others remained healthy.

The evidence certainly indicates that this bacillus is not very effective on any insect yet experimented on outside the order Hemiptera, and that the disease it causes is not likely to be confused with any disease previously described. The growth characters alone, indeed, would serve to distinguish the organism specifically.

TOXIC PROPERTIES.

From one of the early isolation cultures I removed several colonies of the disease bacteria from the surface of the agar, and diffused these in a small quantity of distilled water to serve some inoculation purposes. On immersing young squash-bugs in this infusion, death followed almost immediately. With nymphs somewhat older the effect was not so rapid, but the bugs soon succumbed. Young chinch-bugs, flies, and other insects stiffened as if dead on being immersed from one to several minutes. Many of the hard-shelled insects, if removed immediately on becoming rigid, recover in a few minutes sufficiently to crawl away; but even these die if immersed in the infusion for some time.

The rapid action of these infusions suggested that some poisonous principle was excreted by the bacteria,

for it seemed impossible that an effect so marked could result from any circumstances concomitant to the mere presence of the bacteria in the water. Nevertheless, similar experiments were made with other active aërobic bacteria; but in these infusions no such marked or permanent effects could be induced.

In general, soft-skinned insects were much more readily affected; and the yellow-necked apple-tree caterpillar, *Datana ministra*, proved to be an excellent subject for experimentation. I give in detail a record of the effect on one of these larvæ, as made by Professor Forbes: The larva was dipped for ten seconds in a strong infusion of the bacteria, and then removed to a piece of filter paper for observation. On removal it was quite rigid, but in two minutes there were slight signs of life, and in three minutes it was wriggling and tossing, continuing these incoherent movements until after the fifth minute, when it lay quietly upon its back. Six minutes after removal from the infusion, the larva ceased entirely to respond to touch, and was apparently dead. It was kept for twenty-four hours more, but gave no evidence of recovery. Such insects as squash-bugs, flies, etc., often make characteristic movements when about to succumb to this poison; and in the stiffness that finally ensues, the legs are often closely drawn together. As mentioned before, many insects will recover from the effect if removed as soon as they become stiff. I shall have further occasion to refer to this stiffness produced in insects previous to death, and as it is doubtless analagous to certain effects of heat, cold, etc., we may conveniently employ for it the term toxic rigor.

In order to avoid any possibility of confounding with these toxic phenomena those incident to drowning, a number of water-beetles (*Dineutes discolor*) were secured for experimentation. It was first ascertained that a water-beetle easily lives in pure bouillon many hours—thirty in the case recorded; and after such a length of

time decomposition would have advanced considerably. In dilute squash-leaf decoction this beetle also survives an immersion of many hours; and it is therefore evident that the ingredients which might be dissolved from agar cultures in making the infusions would play no part in the result. In all subsequent cases the infusions were obtained from agar cultures in slanting tubes, or from the Petri dishes, by adding a small quantity of distilled water, and then with the needle diffusing in this some of the bacterial growth. This infusion was then transferred to a deep Petri dish.

It is probably well to give in detail the results of a few experiments with this water-beetle.

(*a*) Three minutes after immersion in an infusion from an isolation culture the beetle became somewhat sluggish, although it made a few rapid dives when touched, and in ten minutes no movement could be induced. After remaining thus immersed five minutes longer, the beetle was transferred to filter paper. Ten minutes later there was slight sign of revival; but this was only temporary. The insect in this case was immersed in an infusion from pure cultures.

(*b*) In seven minutes rapid diving about the vessel had ceased; in nine minutes there was only a slight movement of the limbs; and in fifteen minutes the beetle was apparently dead. It was kept in the infusion for fifteen minutes after the toxic rigor was produced, and was then removed to filter paper; but there was no recovery.

(*c*) A tube culture was sterilized by exposing it to a temperature of 125° F. for one hour on two successive days. An inoculation from this tube showed that it was perfectly sterile, and an infusion was then prepared, in which a beetle was placed. Sluggishness was manifest in seven minutes, and in fifteen minutes there was no sign of life. Fifteen minutes thereafter the insect was removed to filter paper; but the only sign of recovery was a temporary twitching of the limbs.

The above results and a few others are briefly indicated in the table below.

TABLE VI.

No. of experiment.	Source of the infusion.	No. minutes required to produce toxic rigor.	Total period of immersion.	After effect.
a	Isolation culture.....	10	15	Slight temporary movement.
a^1		12	20	No recovery.
b	Pure culture from *a*	15	30	No recovery.
b^1		36	50	Temporary movement of limbs.
c	Sterile culture.....	15	30	Temporary movement of limbs.

Parallel with the above, I also tested a bacillus isolated from the tissues of a diseased grasshopper out of the lot from Colorado. Two beetles were used. One of these remained healthy for several hours, and no further observations were made. The other was kept in the infusion for a much longer period, and at the end of seventeen hours it was still living, though quite sluggish. In a few hours more death ensued.

A comparison of these two groups of tests gives abundant evidence that the squash-bug bacillus produces by its growth some toxic compound which acts with considerable rapidity even on hard-shelled insects. It is well known that many bacteria produce by their growth organic compounds of one kind or another, and it is well to mention in this connection that while a water-beetle will live for more than a day in fresh bouillon, death occurs in a few hours if the bouillon has "gone bad," although it may be subsequently sterilized. The products of such decomposition are thus to a slight degree deleterious to the water-beetle.

In the above table the record of experiments seems to indicate that the isolation culture was slightly more effec-

tive than pure cultures made from it. To determine if the organism was thus attenuated by continuous growth in the laboratory, ten successive cultures were made, the first proceeding immediately from an isolation culture, and the growth in each tube being permitted to mature before the succeeding one was inoculated from it. The first series was kept for one month, and the second series was prepared from this just two days before the experiment was to be made. At this time water-beetles were not procurable, and adult squash-bugs were necessarily used. The latter are rather difficult to operate upon, and recover more readily than water-beetles; but the relative activity of these solutions could be tested by a comparison of the time required to produce the toxic rigor. The results are indicated by the following table.

TABLE VII.

Number removes.	Number of Minutes Required to produce Toxic Rigor.				
	Cultures one month old.	Culture two days old. Successive trials with the same infusion.			Average.
		First.	Second.	Third.	
2	5½	6¼	6½	8½	6.7
3	2½	2½	14½	5½	6.3
4	14½	22½	7¼	5¼	12.3
5	26	9½	4	4¼	10.9
6	4	6¾	8½	6½	6.4
7	7	5½	13½	7¼	8.3
8	7½	6	7½	6¼	6.8
9	4	4½	9½	5¼	5.8
10	7¼	8	7½	4¼	6.8

This record seems to indicate that there is no deterioration in the properties of this organism when grown successively on agar cultures. The individual variations in the above table are probably due entirely to a difference in the resistance of the bugs themselves.

Chemical analyses of the pathogenic principle produced by this bacillus have been kindly undertaken by Professors A. W. Palmer and H. S. Grindley, of the University of Illinois.

MICROSCOPIC CHARACTERS.

Slides of this organism direct from the insect, if properly stained, show a short bacillus, single or in pairs, usually 1.2-1.8μ×0.6-0.8μ. The young bacilli are usually homogeneously stained, but the adult and dividing cells often stain more densely at the poles, frequently leaving the middle part entirely clear (see Plate XXVII., Fig 1). Every gradation from one to the other occurs abundantly in a single preparation. This so-called belted appearance of many bacteria has been written about at length and speculated upon by Billings* and others. In this bacillus the appearance is often less truly belted than the figures of Billings would indicate for the germ of hog cholera, and the line of demarcation between the stained and the unstained portion is less marked. With a magnification of about five hundred diameters, this unstained portion has much the appearance of highly refractive spores; but higher magnifications easily demonstrate the true nature of these conditions. As far as I have observed, the belted appearance does not appear in preparations made from cultures; and in the latter the rods are uniformly shorter. The organism as obtained from agar cultures stains well in acid fuchsin and in Hoffmann's violet. Slides direct from the insect give excellent results with a glycerine solution of violet aniline†, the only difficulty with the latter being that the preparations fade with age.

*"Swine Plague, its Causes, Nature, and Prevention." (Bull. Neb. Agr. Exper. Station, Vol. 2, Pt. I., p. 104, etc.)

†For this stain, and for various technical suggestions, I am indebted to Professor T. J. Burrill.

Some of the squash-bug nymphs sick with this disease, or recently dead, were fixed in hot water, hardened, dehydrated, and imbedded in paraffin by the usual process. Sections were then made, generally 6⅔ μ in thickness, with the idea of ascertaining as well as possible the general distribution of the bacteria within the tissues. It is difficult to find stains that will differentiate an organism under such circumstances. On finding that anilines, hæmatoxylins, and combinations of these worked to little advantage, Löffler's alkaline methylene blue was tried with fairly good results. This was used alone, or after previous staining with eosin.

A few hours after the death of an insect, the tissues are so badly broken down that little of interest is to be gained from a study of such specimens. In sections of an individual fixed just at the time of death, the bacteria will be found in great abundance in all parts of the perivisceral cavity and well differentiated from the blood coagulum. (See Plate XXVII., Fig. 2.) The blood, indeed, appears to have been like a pure culture of the disease organism. At this stage, moreover, the adipose tissue and the hypodermis are considerably broken down, and thoroughly penetrated by the parasite. The cells of the cardiac tissue also show the presence of the bacteria; but the structure of these cells and the form of their nuclei have suffered very slight disturbance at this stage. There are very few structures unattacked besides the muscles and the stout walls of the alimentary organs; and surrounding both of these the bacteria are often found in great abundance.

Sections of a very sick nymph, killed probably not more than an hour before death would have occurred from the disease, show little that is different from the preceding, except that the tissues are somewhat more nearly normal. (See PlateXXVII., Fig. 3.) Another nymph of the lot thus prepared was fixed while in a very early stage of the disease, the only sign of indisposition

on the part of the insect being a slight sluggishness, a touch causing it to respond actively. An examination of sections made from this individual showed the bacteria in the blood to some extent (see Plate XXVII, Fig 4) but they were apparently quite as abundant in the hypodermis. In the adipose tissue there was occasionally found a small colony of the bacteria; but here there was at the time no general and uniform distribution. From these results it was quite impossible to decide whether the blood became infected by the entrance of germs through the spiracles, or whether there was direct penetration of the hypodermis. The former would seem to be most natural, and I am inclined to suppose that the fluid of the perivisceral cavity is the seat of first action. On this ground, however, the more marked effect on nymphs, both of squash-bugs and of chinch-bugs would necessarily be explained on a truly physiological basis, rather than on the ground of the more penetrable character of the chitinous outer coat.

TEMPERATURE EXPERIMENTS.

For a further knowledge concerning the conditions of growth of this bacillus and for a guide to any field applications of the organism, a series of temperature tests was planned, by means of which it was hoped to ascertain (1) the period of exposure to various temperatures necessary to kill this organism when mature, and (2) the range of *optimum* temperature relative to its growth and development. In order to secure a mature growth, fresh slanting agar tubes were inoculated, and by means of the water of evaporation the bacteria were distributed as much as possible. The tubes were kept at about 80° F. for two days, when the growth was abundant over the whole surface, and then the exposures were made as indicated in the table. On last removal from the incubator, other slanting tubes of fresh agar were in a similar way inoculated from these, and the new tubes placed under observation for growth developments.

TABLE VIII.

Tube No.	Temperature ° F.	Exposed.		Result from fresh inoculation.
		No. hours.	No. successive days.	
1	125°	½	1	Good growth
2	125°	½	2	Good growth
3	125°	1	1	Good growth
4	125°	1	2	No growth
5	125°	2	1	No growth
6	125°	6	1	No growth
7	115°	1	1	Good growth
8	115°	1	2	Good growth
9	115°	1	4	Slight growth
10	115°	3	1	No growth
11	115°	3	2	No growth
12	115°	3	4	No growth
13	115°	6	1	No growth
14	115°	6	2	No growth
15	115°	6	4	No growth
16	105°	3	2	Good growth
17	105°	3	4	Good growth
18	105°	6	1	Good growth
19	105°	6	2	Good growth
20	105°	6	4	Fair growth
21	105°	24	4	Slight growth
22	95°	24	4	Good growth

Summarizing, then, it appears that this organism will not survive at 125° F. a continuous exposure of two hours, nor an exposure of one hour on more than one day. At a temperature of 115° F. it will withstand an exposure of one hour for several successive days, but no continuous exposure of three hours or more. It survives in great part an exposure of six hours at 105° F. on four successive days, but with a continuous exposure of four days at this temperature the organism is usually killed.

I have not yet had an opportunity to experiment with this disease while keeping the bugs at different temperatures: but such a line of work would be interesting to supplement the above temperature tests, and to advance our knowledge as to the relationship of conditions required by host and parasite. Neither have I yet determined precisely the range of *optimum* temperature for this organism; but such results as are recorded seem to indicate that it is between 83° and 90° F.

SPECIFIC CHARACTERS.

I have carefully compared the biological characters of this squash-bug organism with those enumerated for the various entomogenous bacteria already described; but there is no form with which it agrees in detail. Its action on the insect and its characteristic pathogenic properties seem to be quite distinct; hence I have given to it the name *Bacillus entomotoxicon*, n. sp.

This disease bacillus has also been carefully compared with the organism "normal" to the cœcal appendages of the squash-bug. There is considerable structural difference, and the normal form is cultivated on nutrient media with difficulty. I have secured cultures of the latter on media strongly alkaline; but further details of such work are not included in this paper, as the cœcal form is hardly to be confused with the disease organism.

Bacillus entomotoxicon Duggar.

Occurrence.—In the blood and tissues of diseased squash-bugs.

Morphology.—Short bacilli 1.2–1.8 $\mu \times 0.6$–$0.8\ \mu$, single or in pairs, motile, not producing spores.

Preparations stain well in most of the anilines, the bacilli often staining much more deeply at the poles, consequently showing a banded or belted appearance.

Growth and pathogenic characters.—An aërobic and facultative anaërobic organism, producing on nutrient agar-agar a dirty white colony often characterized by prominent fan-like radiations. Stab cultures on nutrient gelatine give liquefaction on the second or third day, soon assuming the shape of an inverted lamp chimney, and after standing one month the gelatine is colored wine-red. Milk is rapidly coagulated and the coagulum in great part dissolved, the odor attending this growth on milk being exceedingly vile. Nitrates are not reduced. It grows well at living-room temperature, but is easily killed by exposures to high temperatures.

The infected insect becomes sluggish a few hours before death, and at death it is slightly darker and softer. After death the insect is slightly swollen, darkens rapidly, and soon contains only a mass of gruel-like fluids.

Sterile or active infusions from the growth on agar contain a principle toxic for many species of insects, as shown by temporary immersion.

SUMMARY.

Under the specific description are summarized the leading facts relative to the structure of this organism and to its growth on the usual culture media. It is necessary to add a few brief statements embodying some of the results of general œcological interest.

Bacillus entomotoxicon is the cause of a characteristic disease of the squash-bug, first observed as an epidemic among bugs in a laboratory breeding-cage.

Both laboratory and field experiments show that the disease is readily communicated to healthy squash-bugs by contact with the fluids of infected insects, nymphs being more easily affected than adults.

Fresh agar cultures of the bacillus are effective as sources of infection.

The disease may be communicated to young chinch-bugs either from diseased insects or from cultures; but adult chinch-bugs are strongly resistant.

With the grubs and other larvæ hitherto experimented upon, external applications of infection material have given no successful results.

Infusions from the growth on agar contain an active principle which kills many insects after a very short period of immersion.

Sections of diseased squash-bugs show that the bacillus is present in the blood at all stages of the disease. The hypodermis, adipose tissue, and cardiac tissue are also early affected. When death ensues the body fluids are like pure cultures of the disease organism; and accompanying saprophytic germs are seldom found.

ACKNOWLEDGMENTS.

To the Director of the State Laboratory of Natural History, Professor S. A. Forbes, I owe many thanks for the opportunity of working upon the disease herein discussed, as it is to him that the Department of Insect Disease Work owes its origin. I would also express my thanks to Mr. W. G. Johnson for valuable assistance with various entomological details.

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The list appended deals only with bacterial or so-called bacterial diseases of insects, and in it are included all works and articles to which I have had access, together with the majority of references definitely given in these

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EXPLANATION OF PLATES.

Plate XXVII.

Fig. 1. *Bacillus entomotoxicon* from the blood of a diseased squash-bug. Zeiss $^1/_{12}$ hom. imm., oc. 4, tube length 205 mm.

Fig. 2. Distribution of bacteria in the hypodermis and fatty bodies of a squash-bug at the time of death: *c*, cuticle; *h*, hypodermis; *m*, basal membrane; *a*, adipose tissue much degenerated. Zeiss E, oc. 2, tube length 155 mm.

Fig. 3. Distribution of bacteria in the blood and tissues of a squash-bug an hour or more before death: *h*, hypodermis; *m*, basal membrane; *p. c.*, extension of perivisceral cavity; *a*, adipose tissue. Zeiss E. oc. 2, tube length 155 mm.

Fig. 4. Longitudinal section of the dorsal vessel, or heart, of a squash-bug slightly sick, showing distribution of bacteria in the blood.

Plate XXVIII.

Fig. 1. Growth of the *Bacillus entomotoxicon* on nutrient agar containing a small amount of squash-leaf decoction.

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* All figures were drawn with the aid of a Zeiss camera lucida.