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Article IV.—Studies on the Contagious Diseases of Insects.

By S. A. Forbes.

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ERRATA.*

Page 5, line 3 of table, second column, for 39 read 38; line 6, second column for 121 read 120.

Page 9, line 17, for *conjunction* read *conjugation.*

Page 21, line 13, for *Ricciaciæ* read *Ricciaceæ.*

Page 67, line 17 from bottom, for *fraligifolia* read *fragilifolia.*

Page 123, line 4 from bottom, and page 126, line 1, for *Tricholeæ* read *Trichocoleæ.*

Page 126, line 2, for *Tricholea* read *Trichocolea.*

Page 177, line 16, for *Lecythia* read *Lecythea.*

Page 333, line 1, after Tachidius add Lilljeb.

Page 338, under Daphnella brachyura, line 16, insert *Hab.*—Massachusetts (*Birge*), Minnesota (*Herrick*).

Page 340, line 5, for *Scapaoleberis read Scapholeberis.*

Page 389, line 7 from bottom, for *carpogonium* read *sporocarp*; lines 9, 12, 15, for *oögonium* read *carpogonium.*

Page 391, line 1, for *Cessatii* read *Cesatii.*

Page 400, line 4, for *Myceliumin conspicuous* read *Mycelium inconspicuous*; line 14, for *coleosporium* read *Coleosporium.*

Page 401, line 9, for *connatus* read *connata;* line 12, for *Taraxicum* read *Taraxacum.*

Page 408, line 15, for *macrocarpa* read *macrospora;* line 18, for *Hypohyllous* read *Hypophyllous.*

Pages 470 and 471, head of column 11, for *cyprinella* read *cyprinellus.*

Page 503, lines 8, 14, and 17, for *cyprinella* read *cyprinellus.*

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* For additional errata see page 247.

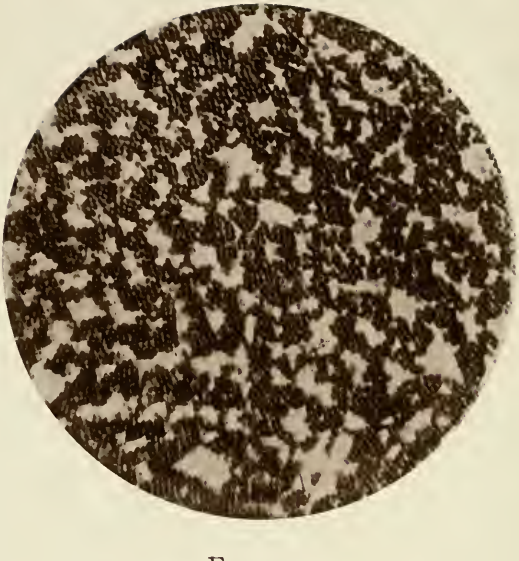

FIG. 1.

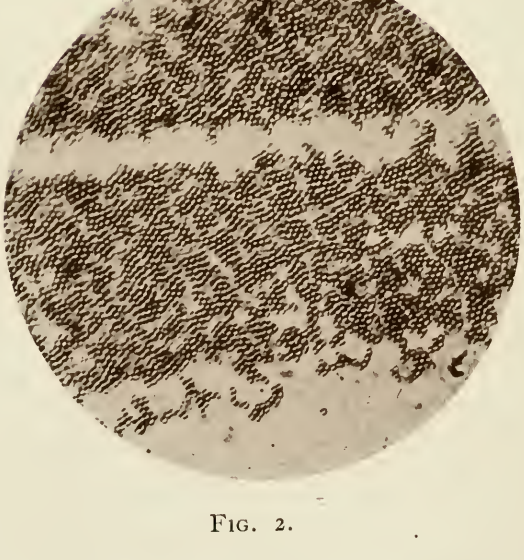

FIG. 2.

Culture of MICROCOCUS from diseased Cabbage Worm (*Pieris rapæ* L.), in test tubes of sterilized beef broth, commenced October 20, 1883 (see p, 275). Preserved over winter in plugged test tube, and slides mounted April 10, 1884, in carbolized water, after staining with brown aniline.

Photographed with lamplight by Dr. H. J. Detmers, with Spencer $\frac{1}{10}$ homogeneous immersion, × 1000.

Both figures are from different parts of the same slide, differing only in focal adjustment,—figure 1 being a "positive," and figure 2 a "negative."

Article IV.— Studies on the Contagious Diseases of Insects.— I.

By S. A. Forbes.

Since August, 1883, the writer has used such opportunities as came in his way for observation of the diseases of insects, and for more or less careful and systematic work upon them, directed especially to the point of artificially propagating them for the destruction of injurious insect species. While these researches are not by any means completed, lacking especially critical study of the bacterial forms dealt with, as botanical species, and imperfect also on the side of field experiments on a large scale, I have thought them worthy of present report as a contribution to progress on a difficult but interesting subject, especially as opportunity for further continuance of some of these studies may not soon return.

My main object has been experimental and economical, and I have adopted such methods of study as seemed to me to offer the simplest means of surely ascertaining whether some of the common diseases of our insects were of bacterial origin, whether their germs were readily and conveniently cultivable, and whether such cultures could be used to convey the original affections to healthy insects.

This will serve to explain what may seem to some an excessive reliance on fluid cultures,—much more convenient for my purpose in these preliminary studies than "solid cultures" with gelatine films or tubes, and quite conclusive as to the identity of the forms dealt with, if the cultures are often enough repeated and the results are closely scanned.

Without attempting at this time to summarize the literature of the subject,—scattered and chiefly fragmentary, except as relates to the silkworm and the honey-bee,—I give here only a brief account of my own earlier notes and observations.

The appearance of what seemed to be an epidemic of contagious disease among the chinch bugs of Central Illinois in the latter part of the summer of the above year, gave rise to an article on this subject, published in 1883 in my first report as

State Entomologist of Illinois (pp. 45–57). This article contained an account of a considerable series of microscopic observations on the fluids of chinch bugs apparently affected with disease, and described some successful attempts at the culture of the Micrococcus found invariably characterizing this insect affection. Time failed for further experiments, and the chinch bug has since been so scarce in my vicinity that no further opportunity has offered to complete the study of the subject. The observations made amounted to a practical demonstration of the occurrence of a "germ disease" in this insect species, identified the germ as a Micrococcus, since described as *Micrococcus insectorum*, Burrill, and proved that this was easily and freely cultivable in beef broth. The Micrococcus was shown to have its seat in the alimentary canal of the insects, occurring most abundantly in the posterior part of the same, to infest pupæ and adults more seriously than the younger stages, and to have the apparent effect to retard the development of the brood as well as to destroy a large percentage of them before they reached maturity. This disease was apparently the representative of *flacherie* or *schlaffsucht* in caterpillars, as described by previous authors and in the following pages.

Next there appeared early in August, 1883, in our breeding cages of *Datana ministra* (the yellow-necked apple caterpillar), an outbreak of disease characterized by the occurrence, at first in the alimentary canal and later in the blood, of immense numbers of micrococci of a form very different from the above, and evidently quite readily conveyed from one insect to another. Elaborate studies of this disease were made during the remainder of the season and the following spring, the bacteria associated with it were repeatedly cultivated with success in animal infusions, and several experiments were made to convey the disease by their means to still healthy larvæ. Tubes of the culture fluids were sealed up for preservation over winter, their contents were cultivated again in June, 1884, and the resulting cultures were used to infect the food of larvæ of *Mamestra picta*, with the hope of thus reproducing the original disease of the Datana larvæ of the preceding year.

Parallel with these experiments was a similar series made on a frightfully contagious and destructive disease of the European

cabbage worm (*Pieris rapæ*), first observed by us at Normal, September 11, 1883. The bacterial character of this disease was ascertained, many attempts at cultures were made, some of them successful, and the possibility of conveying the disease to a distance by means of affected cabbage worms was tested by us in Western Illinois and Iowa. Many of the observations and experiments relating to *flacherie* in this insect were repeated by me in 1884, and in the early summer of 1885 admirable photographs of several of the slides were made for me by Dr. H. J. Detmers, of Champaign.

"Jaundice" of the silkworm appearing in an experimental nursery of this species, under the charge of Professor Burrill at the State Industrial University, at Champaign, in June, 1884, an opportunity was afforded me to study this affection. Many successful cultures were made of the bacteria involved, and several experiments were undertaken for the infection of healthy cabbage worms with the contagion from these artificial cultures. Succeeding in the laboratory, these experiments were carried into the field, and attempted on the large scale of actual practice.

An epidemic of muscardine appearing in certain breeding cages of the forest tent-caterpillar (*Clisiocampa sylvatica*) in June, 1884, this disease was studied by us as there illustrated, and connected more or less certainly with a destructive epidemic of the preceding year, which had swept away vast numbers of that species under my observation in Southern Illinois.

With the exception of the *flacherie* of the chinch bug, these observations have not hitherto been anywhere fully reported, although brief notices and general accounts of a more or less popular character have been printed in the scientific journals and in some economic publications.

The chinch bug observations were published, as already mentioned, in the Twelfth Report of the State Entomologist of Illinois, the species of Micrococcus concerned having been previously described by Prof. Burrill in the Report of the Trustees of the Illinois Industrial University for 1882, and in the "American Naturalist" for March, 1883.

A brief preliminary paper on *flacherie* of Datana was read to the entomological club of the American Association for the

Advancement of Science at its Minneapolis meeting in August, 1883, and of this a synopsis appeared in the "Canadian Entomologist" for September, 1883. In the "Prairie Farmer" (Chicago) for October 6, 1883, and in "Science," also, for October 5, 1883, brief notes occur with reference to this disease in the cabbage worm.

In the Transactions of the Illinois State Horticultural Society for 1883 (printed February, 1884) is a somewhat elaborate paper on the Contagious Diseases of Caterpillars, read before this Society December 18, 1883, giving a general and rather popular account of the character of the work done by me on this subject, up to that time; and a still more elaborate paper (never published) was read before the State Natural History Society of Illinois, at its meeting in Peoria, July 8, 1884, in which a classification of insect diseases was presented, and a full *résumé* of methods and results, up to that date, was given. At a meeting of the State Horticultural Society, held at Champaign, December, 1884, I added some further items relating to cultures and experiments, especially those affecting the cabbage worm, and these notes were published in April, 1885, in the Transactions for the year preceding.

It is my purpose, in this paper, to present the principal results of the above studies,— both the successful and the unsucessful issues,— the latter so far as they have any significance or value.

Disregarding the chronological order of my observations, I shall first discuss *flacherie* of the cabbage worm, and jaundice of the silkworm, with experiments upon the former insect with the artificial cultures derived from the latter. I will then take up the longer and more complicated record of *flacherie* in our Datana larvæ and the experiments drawn from it, and will conclude with a brief account of the *muscardine* of the forest tent-caterpillar.

EUROPEAN CABBAGE WORM *(Pieris rapæ, L.)*

In studying experimentally an insect disease, it is necessary, in the present state of our knowledge, (1) to determine precisely the symptoms and character of the disease itself, in order that it may be subsequently recognized with certainty; (2) to

learn whether it is characterized by bacteria; and (3) whether it is practically contagious. Determining these questions affirmatively, (4) cultures of the bacteria must be made artificially, and (5) these cultures must be used to produce, in healthy insects of the same or other species, a disease characterized by the symptoms and results of the original affection. It is further desirable that (6) second cultures should be prepared from these cases of disease artificially produced, in order that a strict comparison may be made of the bacteria concerned, as they occur both in the bodies of the insects and in artificial culture fluids.

I propose to take up these points *seriatim*, (first with respect to *flacherie* in the cabbage worm), presenting separately the facts bearing upon each, only premising that the proof of one proposition is sometimes partly contained in the data relating chiefly to another, so that some repetition will be necessitated by this mode of discussion; but this disadvantage will doubtless be found insignificant, compared with the gain in clearness and cogency.

Description of Flacherie in the Cabbage Worm.

In this insect *flacherie* is distinguishable with great ease and certainty by conspicuous external symptoms, the color alone of affected larvæ being, in fact, entirely characteristic and unmistakable. The natural color of a healthy cabbage worm is a light lively green, sometimes slightly tinged with yellowish, but without any approach to an ashy or milky hue. As the first symptom of *flacherie*, however, the larva commences to turn pale, this paleness increasing more or less rapidly until the color is almost milky white, only slightly tinged with greenish. This discoloration is uniform and simple, no other tint usually appearing until after death. Then, however, the color deepens to a sooty gray, commonly uniform, but sometimes appearing first about the center of the length of the larva. Occasionally this deeper color appears a little before death, but it is not then of equal depth over the whole surface.

In the actions of the insect there is little to indicate any change of state, except a gradually increasing sluggishness,

slowness of movement, and loss of appetite. These are later to appear than the pale discoloration above mentioned, and even shortly before death a larva may show considerable impatience if roughly handled. When the disease is well developed, the caterpillar is very feeble, and will remain motionless for a long time; or if it attempt to crawl where some strength is needed, as horizontally on a vertical surface, it may lose its hold with its jointed limbs and cling only by its central prolegs, the fore and hinder parts hanging limp and helpless at right angles to the remainder of the body.

Most commonly an escape of fluid from the vent is among the earlier symptoms of the affection, at first greenish or whitish, and later a dirty gray, or even a chocolate brown. Rarely this fluid exudes also from the mouth. The amount of it is usually sufficient to stain considerably the surfaces over which the larva crawls: but sometimes this symptom is wholly absent. Occasionally the stomach is found empty after death, but almost invariably it is well filled with food, much of which has not yet lost its native color, digestion being, in fact, evidently suspended during the course of the disease. I have found in only a single instance an appearance of bubbles of gas in the alimentary canal, such as Pasteur describes in the *flacherie* of the silkworm. Usually the mass of the alimentary contents seems to lie inert in the stomach, undergoing neither digestion nor decay.

The color of the fluids of the healthy larva is a very pale transparent green, the blood containing only lymphoid corpuscles in greater or lesser number; but if a proleg of a diseased specimen be snipped off, and a cover glass be pressed against the cut surface, the droplet exuding will be of almost milky whiteness, or, in the latest stages of the disease, a dirty gray. Rarely, where there has been much escape of fluid from the vent, the juices of the larva will be thick and scanty, so that it requires some pressure to force out a very small quantity. If a minute droplet of the milky fluid obtained by snipping off a proleg be examined under a high power of the microscope, it will found to contain innumerable myriads of very minute spherules, varying in diameter, according to the individual, from .5 μ to 1 μ. Usually their average size does not surpass .7 μ. It is

the infinite multitude of these which gives to the fluids of the diseased caterpillar their milky look, and likewise, unquestionably it is they which cause the ashy appearance of the surface, the skin being thin and delicate, so that the color of the fluid contents shows through. The diseased blood is so thick with these minute corpuscles that little else can be ordinarily seen in it. Sometimes, however, degenerated lymphoid corpuscles of the blood will be noticed, recognizable by their size and spherical contour, but differing from the normal corpuscles in their darker tint and coarsely and irregularly granular structure. These darker, granular corpuscles are always dead, no longer exhibiting amœboid movement, and have usually a spherical form. Not infrequently *débris* of the fatty bodies is apparent in the form of large irregular cells, floating freely in the fluid, but these cells themselves will be found to contain immense numbers of the minute spheres already mentioned. In fact, if a little portion of the soft remnant of the fatty bodies be removed, spread upon a cover, and examined with a power of a thousand diameters, it will be seen that the cells of these organs are the seat of an extreme degeneration, the entire contents of many of them being wholly replaced by the spherical granules mentioned above. Occasionally a cell containing a nucleus will be found, but more commonly all distinction of contents has disappeared.*

* As an example of the condition of the fatty bodies, I will describe those of a larva examined October 9, whitish in color and nearly dead, making little effort to escape. A droplet of the blood exuding from a small cut made in the back was alive with the minute spherules already mentioned, and contained also noticeable numbers of dead blood corpuscles in a dark, spherical, granular condition, together with a few unaltered examples still capable of amœboid movement.

A fragment of the fatty bodies examined. consisted chiefly of pale spherical cells, 1.5μ to 7.5μ in diameter, resembling oil globules, except that they had not the high refractive index of fat. A few of these globular cells were very pale and indistinct, the contents very indefinitely granular and often with a large spherical nucleus likewise very pale; but most of them were more or less completely filled with dancing spherules, slightly different in size in different cells, these differences having, however, no relation to the proper size of the cells. Sometimes there were not more than twenty-five or thirty such granules in an optical section of a large cell, the con-

If the body of a diseased larva be cut across and a cover glass be pressed against the cut end of the intestine, or, still better, if the larva be opened lengthwise, the stomach removed and laid open separately, so that a droplet of the pure contents of the alimentary canal may be obtained, the fluid portion of these contents will be seen to swarm with infinitesimal granules identical in appearance with those found in the blood, except that they are, on an average, often appreciably larger and are occasionally more or less oval in outline. These same forms may also be found in the fluid excreta escaping from the vent of the still living larva. If the specimen has been dead some time, so that the sooty discoloration of the surface has occurred, the fluids both of the alimentary canal and of the body at large will often be found to contain, besides myriads of the above spherules, various other forms clearly recognizable as septic bacteria,—among these, members of the genus Bacterium, easily distinguishable by their oval form and by the manner in which they actively propel themselves across the field of the microscope. Rod-like bacilli may also appear in the fluids at this time, equally active, and evidently moving by means of flagella, especially in the vicinity of the bubbles of air which may be included in the fluid under the cover glass. Occasionally these latter bacterial forms may be found in smaller numbers even before death, very rarely in the perivisceral fluids, but not very uncommonly in the contents of the alimentary canal. Still they are infinitely less abundant than the Micrococcus-like spheres already mentioned, even long after the death of the larva.

The most characteristic *post mortem* phenomenon is the rapid softening, decay, and deliquescence of the body, the whole of which may be converted, in an hour or two after death, into a dirty fluid mass which the rotten skin is barely sufficient to hold together. This breaks at a touch, allowing the fluid contents to escape.

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tents being otherwise fluid. Many of the cells were not full, areas occuring which the dancing particles did not invade. Occasionally an unaltered nucleus would be seen in the midst of the corpuscular contents of the cell. The fat globules intermingled were easily distinguished from the above cells by their very different refracting power, and were always free from the spherical granules. They were less than half as numerous as the pale cells. The average size of the granules was not far from .66 μ.

The Characteristic Bacteria.

As implied in the foregoing, I have no doubt that a large percentage, at least, of the minute spherical granules abundant in the fluids of the body and alimentary canal of the diseased larvæ are genuine bacteria, belonging to the genus Micrococcus I cannot hope to convey verbally the same conclusive conviction of this fact which I have myself derived from long study of these little forms under a great variety of conditions, and the preparation and examination of a multitude of slides, both recent, and permanently mounted. These latter were sometimes unstained, and again stained with a considerable variety of aniline dyes,—brown, blue, violet, and magenta. Several successful cultures have also assisted to confirm this view, the products of the cultures being unmistakably the form originally taken from the caterpillars under experiment.

In form the micrococci of the cabbage worm are usually strictly spherical, although in the alimentary canal a patch will occasionally occur in which they are of a slightly oval outline. The micrococci of the fluids of the diseased larvæ seen in the field of the microscope are mostly separate spheres, but a considerable percentage of them are attached in pairs, as if in process of division. Rarely a short chain of four, six, or eight may be seen. In the stomach they occur not infrequently in compact patches or zoöglœa-like masses. In size the individuals vary from .5 μ to 1.25 μ in diameter, the small forms being those in the blood and the larger those in the stomach. Individual larvæ differ, in fact, with respect to the size of their micrococci, in some the average of those found in the blood being not far from .75 μ to 1 μ, while in others they barely reach .5 μ. Commonly, those of the stomach average 1 μ.

In addition to the direct evidence above adduced, the close resemblance of these corpuscles to those occuring in other larvæ affected similarly to the cabbage worm, in which the bacterial character was even less obscure, gave indirect and cumulative evidence with respect to the nature of those forms in the cabbage worm. Their reaction to the usual staining fluids was such as the hypothesis of their bacterial character

would require. Although staining with some difficulty, many slides were prepared in which the individual bacteria were beautifully stained and distinctly differentiated from an uncolored film, by brown aniline, methyl violet, and magenta.

Although several of the attempts at artificial culture were abortive, and although the cultures resulting were sometimes impure and occasionally doubtful, enough cases of unquestionable success occurred to give full effect to this mode of proof. The details supporting this statement will be given under another head. It is worthy of special remark that in no case did the beef broth in which these cultures were made, although it became densely milky with bacteria, give off the slightest smell of decomposition. Only a faint, indescribable odor was perceptible, but little different from that of the fresh liquid.

To the above we may add the association of these spherules with diseased conditions and with *post mortem* phenomena which could scarcely be accounted for at all, except on the supposition of the bacterial character of these excessively abundant forms.

The proof of the contagious character of the disease in question, next to be adduced, must also be taken as indirect, or, at least, *prima facie* evidence, in the present state of our knowledge, of the living organic character of these multitudinous particles,—the only forms present which could in any way be connected with the disease as agents of the contagion.

Contagious Character of the Disease.

Most of the considerations brought forward in the preceding section apply with some force to the subject of this, for if the fluids of the diseased and dead larvæ swarm with micrococci so minute (these appearing in the blood long before death), and if these are shown to escape from the body by way of the excrement and the fluids exuding from the vent, the presumption is strong that the disease which they characterize would be conveyed to healthy individuals by their instrumentality. But we must look for proof of contagion chiefly to the conditions of the occurrence of the disease, to the phenomena

of its spread, and especially to the results of experiments for conveying it artificially to localities or regions where it had not before appeared.

That this affection, or one very similar to it, attacks the cabbage worms of the old world, is made likely by a chance remark in Curtis's "Farm Insects" (p. 96), where he says of several larvæ of an allied species, *Pieris brassicæ:* "On the 20th they appeared healthy, but inclining rather to a yellow color; it rained during the night, and on looking at them in the afternoon of the following day, I saw they had removed to a leaf, to which they stuck by four of their hinder legs, and, to my surprise, they were of a dirty color, and rotten, the skins being lax, and lying just as the wind blew them about. I found they only contained some cream-colored fluid, a portion of which was scattered upon the leaves."

In this country the disease seems to have been first noticed in the vicinity of Washington, in 1879, although little attention was paid to it, and its bacterial character was not then ascertained. In Bulletin 3, of the United States Entomological Commission, (pp. 69, 70), Dr. Riley remarks, while discussing some experiments made with yeast on the cabbage worm:

"An incident connected with these experiments which I made is, however, well worthy of being mentioned, because it shows how very easily single experiments may lead to false hopes and conclusions. A certain proportion of the last-named larvæ — the proportions differing in the different lots treated — perished before or while transforming to the chrysalis state. They became flaccid and discolored, and after death were little more more than a bag of black putrescent liquid. I should have at once concluded that the yeast remedy was a success, had I not experienced the very same kind of mortality in previous rearing of this larvæ, and had I not, upon returning to the field from which the larvæ in question were obtained, found a large proportion similarly dying there."

No other notices of it have occurred in my reading, previous to those of its appearance in Illinois, already mentioned (October 5, 1883). That it did not occur at Normal in 1882 is made certain by the fact that the cabbage fields there were frequently visited in autumn by myself and my assistants dur-

ing the progress of a series of experiments with insecticides upon the cabbage worm, and that nothing of the sort was seen by us.

When first noticed there, its distribution was peculiarly irregular. In certain small fields, for example, not one half mile distant from those in which the disease was raging violently, affecting one fourth to one half of the worms in sight, not a single dead larva could be found by very careful search. A few weeks later (October 4), larvæ in these fields were suffering as severely as the others, 20 per cent. of the worms, on an average, showing signs of illness.

September 27, at Rosehill, near Chicago, I visited fields in which, although the worms were fairly abundant, I could not find a single diseased larva during a careful examination of more than a hundred individuals; while across a road and a half mile away, the disease was fully at work in four adjacent fields, and fully one fourth of the worms had been attacked. These were in all stages of the disease, many of them being dead and rotten. The identity of the affection with that observed at Normal was established by careful microscopic examination.

From correspondents to whom I had described the cabbage worm mortality at Normal, I received various reports. Prof. A. J. Cook, of the State Agricultural College of Michigan, wrote me, October 2, that about 10 per cent. of the cabbage worms near Lansing were affected by it. On the other hand, Prof. Lintner, State Entomologist of New York, informed me, November 3, that it had not been noticed with him. Dr. E. R. Boardman, in Stark county, sixty miles northwest of Normal, reported, September 29, that the cabbage worm was there very destructive, but that no appearance of the disease in question was discoverable. October 5 he repeated this observation, but on the 13th of that month he finally found a very few affected larvæ.

D. S. Harris, of Cuba, in Fulton county, nearly south of Dr. Boardman, first wrote me on the 13th of October that no disease had appeared among the cabbage worms about his place, nor at adjacent towns, as he had learned by careful search and inquiry, but on the 25th of the month he wrote: "That disease

attacking the cabbage worm has made its appearance in Cuba at last. On the 21st I found one full-grown worm sick (head downward), and in about five hours it was dead and decomposed, and several others were affected. To-day it is a difficult matter to find a sound worm on the plants, while the remains of dead worms are numerous."

From Prof. G. H.French, at Carbondale, and Mr. Frank Earle, at Cobden, I learned that the disease had not appeared in Southern Illinois as late as October 29, nor did it occur there during the season. From Champaign, east of me, Prof. Burrill wrote me, October 25, that he had not yet seen any of it in his small garden patch of cabbages, although watching carefully for it; but that an intelligent student had described it as occurring in fields near the town.

In Iowa, to the westward, it seems not to have occurred spontaneously that year, the only appearance of it noted by Prof. Osborn, of the Agricultural College of that State, being the result of an experiment, the material for which I furnished him from Normal. Wherever it once occurred it continued to prevail throughout the season, as far as our observations went.

The facts clearly and positively negatived the supposition that there was anything in the weather or local conditions to explain either the presence or the absence of the disease, and all bore out the hypothesis of a gradual progress from the east westward. The same phenomena of irregular local distribution were manifest the next year (1884). In certain large fields almost daily observed, it was impossible to find a single diseased larva at a time when, half a mile away, the cabbage worms of small patches had been almost wholly destroyed, their blackened bodies, or the shriveled remnants of the same, being scattered everywhere on the leaves.

I may say, incidentally, that the effect of the epidemic in limiting the ravages of the worms, was very evident last year. For the first time in several seasons large fields of late cabbage were brought to full maturity without the loss or serious damage of a head.

From the foregoing the conclusion is unavoidable that all the circumstances of the natural occurrence and spread of the disease are consistent with the hypothesis of its contagious character, and wholly inconsistent with any other.

Two attempts were made to convey the contagion by means of diseased larvæ to localities not reached by it,—one lot being sent October 3, to Dr. Boardmam, at Elmira, and one to Prof. Osborn, at Ames, Iowa. The experiment of Dr. Boardman was not wholly satisfactory, for the reason that through an unfortunate delay of the package the worms which I sent him did not arrive until October 22, at which time the disease had appeared spontaneously, in a small way, in his vicinity. Nevertheless he selected, October 23, two lots of twenty-five worms each, all perfectly healthy to appearance, fed them regularly, but exposed all of them to the contagion by enclosing them in two boxes with the dead and sick caterpillars which I had sent him. At the same time he secured ten healthy larvæ in a box by themselves and kept them free from infection. The latter lot all pupated without accident, but were not followed further. The first two lots commenced to show symptoms of disease on the fifth day, and by the eighth day all of both lots were dead, except three, only one of which finally reached pupation. Even this pupa, in fact, afterwards died and decayed. By this time, however, the disease was so violently raging in the open fields that no great value can be attached to this experiment, especially as the fluids were not microscopically examined.

The material sent Professor Osborn, of Iowa, including dead and dying worms and a mounted slide of the micrococci, arrived October 5, and two cabbage heads were at once infected. On the 7th one of the worms "had evidently succumbed to the disease." The gathering of the cabbages under observation during the temporary absence of Prof. Osborn necessarily interfered with the further progress of the experiment, but he collected such worms as he could from the stumps and fed them in confinement. A number of these larvæ died, and December 28 he wrote me that he had "found micrococci in a number of sick and dead cabbage worms, which must certainly have taken the disease from the ones sent."

Although these experiments, taken alone, could scarcely be regarded as conclusive as to the contagious character of *flacherie*, taken in connection with the other facts mentioned, we must at least allow them some weight as cumulative evidence.

Artificial Cultures of Bacteria.

Methods of Culture.—The modes of culture used in all the experiments reported in this paper were based, unless otherwise specified, on those of Klein, as described in his paper "On the Relation of Pathogenic to Septic Bacteria," in the Journal of the Royal Microscopical Society for January, 1883, differing only by modifications which will appear in the description. The cultures were usually made in beef broth (rarely in infusion of cabbage) in test tubes plugged with sterilized cotton, or in ordinary flasks similarly closed. The broth was prepared by boiling lean beef from a half hour to an hour in a porcelain-lined vessel, and then filtering and carefully neutralizing with caustic soda. The tubes, flasks, and cotton were sterilized by heating in a tin oven, over a gasoline stove, for several hours, at a temperature of not less than 275 degrees or more than 300, as determined by a thermometer inserted in the oven. The heat was sufficient to considerably scorch the cotton without actually charring it. While still within the hot oven the tubes and flasks were securely plugged by means of steel forceps freshly heated in the flame of an alcohol lamp, the cotton plugs, from two to three inches in depth, being pushed firmly in. Most frequently the mouths of these plugged vessels were covered with a cap of sterilized cotton, held in place by an inverted beaker also carefully sterilized by dry heat. In charging the vessels with the fluids the plugs were rapidly withdrawn and as promptly returned, the infusions being introduced boiling hot and afterwards boiled for several minutes to destroy any germs which might have entered during the instant before the plug was replaced. It was not found necessary to test the sterilization of the tubes by protracted incubation, as all the check tubes and the stock flasks in which the store of prepared infusion was preserved, remained unchanged throughout the entire season. Neither was any incubator required, the ordinary temperature of the air during the weeks when these experiments were in progress being never below 60° by day, and ranging commonly above 70° both day and night.

When a culture tube or flask was infected with the fluids of a larva, the following process was invariably used. From

small glass tubes, about a quarter of an inch in diameter, pipettes were made in the flame of an alcohol lamp by drawing out each end to a capillary filament, the tips being closed by melting at the time of making. To charge these pipettes, the end of a proleg of a caterpillar was usually cut off with sterilized scissors, the point of the capillary tube broken off with forceps just flamed in an alcohol lamp, one of these points pushed into the cavity of the proleg, and the pipette partially filled by exhaustion of the air from the other end. To introduce the droplet of fluid so obtained into the test tube we invariably, removing first the beaker and the cap of cotton contained within it, carefully forced down through the cotton plug the capillary tube of the pipette containing the infection material, without loosening at all the plug itself. Sometimes the tip of the tube containing the fluid was broken off inside the test tube before the withdrawal of the pipette, at other times the contents were carefully forced out with the breath, pains being taken not wholly to expel the fluid contents of the pipette. After withdrawal of the tube, the cotton plug was grasped with sterilized forceps and slightly twisted within the mouth of the test-tube to close effectually any small opening through the plug which might have been made by the introduction of the pipette. After this the cap of cotton and the beaker glass were restored, and the tube was set aside with a companion precisely like it in all respects, except that it had not been infected. During the latter part of our investigations these check tubes were themselves operated upon with capillary pipettes, distilled water only being introduced, at the time that the experimental tube was infected.

In withdrawing portions of the products of the culture for inspection a similar process was used, the freshly made pipettes being introduced as for infection and partially filled by exhausting the air from the upper end. After withdrawal, the cotton plug was again twisted as already described and the cap returned. The cover glasses used in the examination and preparation of the material, whether this was derived directly from the larvæ or from artificial cultures, were flamed with an alcohol lamp immediately before using, after being thoroughly cleaned by rubbing with a linen cloth. Slides were similarly

treated for study with the microscope. A droplet of the fluid was allowed to flow from the tip of the pipette upon the cover glass, spread in a thin film by means of the capillary glass tube, and either placed at once upon the slide for immediate examination, or laid aside under a glass shade to dry. After drying, if it was desired to stain and permanently mount the specimen, the cover with the film attached was passed repeatedly through the flame of an alcohol lamp, covered for some minutes with a drop of the staining fluid (the glycerine aniline colors recommended by Prof. T. J. Burrill*), and then thoroughly washed with distilled water. The covers thus prepared were often mounted in balsam, but most frequently, at first, in carbolized water in very shallow cells made with white zinc cement, this cement being also used to fasten the covers to the slides. For microscopic study of the material, my principal reliance was a superb $\frac{1}{16}$-inch homogeneous immersion objective, made to order for the purpose by Herbert R. Spencer & Company, of Geneva, New York. This objective was used with Bulloch oculars, giving powers ranging from 500 to 1,450 diameters. Some of the more interesting or difficult slides were also studied under a $\frac{1}{18}$-inch homogenous immersion of Zeiss.

The measurements here reported were originally made by means of an eye-piece micrometer so graduated that with the highest powers used, each space equalled 2 μ, the micrococci being commonly measured in doubles and chaplets. Many of these measurements were verified by repetition with a more finely divided micrometer, the spaces of which, with a power of 1,000 diameters, had a value of .3 μ; but practice with the more coarsely spaced scale enabled me to measure as accurately with this as with the other, and with much greater convenience.

The products and results of the fluid cultures were commonly so satisfactory that I rarely resorted to solid cultures upon gelatine films. A few of these were made, however, but not with the micrococci of the cabbage worm; and they will be described under the head of the Datana larvæ. As I was primarily interested only in the disease and secondarily in the bacteria, cultures on films were less essential to my purpose

* Proceedings Amer. Society of Microscopists, 1883, p. 79.

than if I had wished to discriminate and describe the various forms appearing. I depended upon frequent repetition of the experiments and uniformity of results, rather than upon the more critically exact cultures and continuous observation of current methods with gelatine films and masses.

Culture Experiments.—Concerning our first cultures, the fact should be remembered that one could rarely expect to find a perfectly pure culture in the body of a diseased insect, exposed as it is by way of the food ingested to invasion by bacteria in great variety. I consequently did not find it remarkable that several of our unquestionably sucessful *infections* were not really pure *cultures*, other bacteria developing than those most abundant in the original fluids. For example, in the very first culture made,—one in beef broth begun September 16, when the infection process was very carefully managed without the slightest accident, and when the check tube remained clear indefinitely,—the culture became turbid the following day, and by October 3, was nearly as yellow as cream, with a thick yellowish felt on top and an abundant precipitate. The greater part of the product of this culture consisted of micrococci like the larger of those of the cabbage worm, the spherules, in singles and doubles, averaging 1 μ in diameter; but the surface film consisted largely of a Saccharomyces embedded in the Micrococcus. Individuals of Bacterium also occurred in the slides. The check tube, as already mentioned, was quite clear to the end.

The second culture was still less conclusive and satisfactory. A cabbage worm which, on the afternoon of the 17th September, was noticeably paler than its companions, was isolated and watched. At 9 p. m. it seemed a little stupid, but otherwise unchanged. At 9 a. m. of the 18th, however, it was dead, blackened, and very soft, the contents evidently little better than fluid. These fluids contained two micrococci,—one a larger spherical or slightly quadrate form, 1 μ in diameter, and the other a minute spherule .5 μ to .75 μ.

A small flask of rather weak beef infusion was infected from these fluids in the usual manner, and the next day, the 19th, it was already decidedly milky. Examined, it was found to contain Bacterium and the larger Micrococcus above men-

tioned, a slender Bacillus, and another Bacillus-like form, of which there will be further question hereafter,—a short, broad form with rounded ends and a paler center,—but nothing resembling the smaller Micrococcus. These organisms were quite possibly all septic bacteria, derived from the decaying body of the caterpillar.

The first unmistakable culture of the Micrococcus of the cabbage worm was made October 20, in a test tube of beef broth infected from the blood of a larva about half grown, decidedly pale, but far from dead. The slide representing the blood of this larva is not stained, but is in good condition. There were two bacterial forms visible in it,—a spherical Micrococcus .7 μ in diameter, and, very rarely, a slender Bacillus. The flask in which the culture was made was poured from a stock flask into a sterilized tube from which the plug of cotton had just been removed, plugged again, boiled thoroughly about three minutes and left to cool completely. The blood was obtained by snipping the skin of the back with sterilized scissors, and drawing up with a fresh pipette a little of the thick fluid exuding. The tube was infected in the usual manner, and not examined until two days thereafter, when it was found decidedly turbid, although not extremely so. The Micrococci were strictly spherical, 1 μ in diameter, very uniform and abundant, usually in doubles, but often single. The slides made were excellent and well stained, some violet and some brown. The bacteria differed from their originals only in being somewhat larger. Still they were not larger than the Micrococcus of the cabbage worm is often found, especially in the intestines. The check flask remained wholly clear.

Four other successful cultures of this Micrococcus were made, so similar in all respects to the preceding that it is not worth while to repeat details. It must be admitted, however, that the minute blood form did not certainly reappear in its *original size* in any of my cultures, if we except one case where its numbers were relatively so few (about 100 to the field with a power of 1,000) that it is barely possible that all were introduced in the original infection. This fact is capable of either one of three interpretations: (1) We may suppose that the proof is incomplete that these smallest spherules from the blood

were micrococci at all, notwithstanding their uniform shape, size, and character, and the fact that they were repeatedly distinctly stained; or (2), taking for granted their bacterial nature, we may suppose them insusceptible of culture under the conditions supplied; or, finally (3), we may assume that the conditions of tube culture in beef broth were so different from those occurring within the blood of the insect as to increase the size or even modify the form of the Micrococcus in question. In favor of the latter hypothesis we have the fact of the generally larger size and often slightly oval form of the micrococci found in the intestinal fluids, as compared with those in the blood of the same specimen.

These considerations apply, however, only to the minute blood form, and not at all to the intestinal Micrococcus. This I have cultivated repeatedly with indubitable success in this insect, and, still more frequently, forms indistinguishable from it occurring in other species. I venture to add that the frequency with which certain bacteria, different from the infection material, appeared in the test tubes when these were infected from the cabbage worm, suggested repeatedly the hypothesis of an alternation of certain forms which were in this way frequently connected,—a point on which I shall have more to say when describing the Datana bacteria. Especially was this true of the larger Micrococcus and of the short, broad Bacillus (?) with pale center and rounded ends (here called, for convenience, *Bacillus intrapallens*). The latter, I shall presently show, behaved precisely like a pathogenic form,—giving no odor of putrefaction in fluids swarming with it, killing insect larvæ whose food was treated with it, and certainly multiplying for some days within their living bodies.

The late period at which successful cultures of the cabbage worm Micrococcus were made precluded attempts at artificial infection by their means, and with respect to this particular insect this part of the proof is consequently wholly wanting.

When the evidence is given respecting the reproduction of what was clearly the same disease in other insects, I think that no reasonable doubt will remain that *flacherie* of the cabbage worm may be conveyed through artificial cultures of its Micrococcus.

THE SILKWORM *(Bombyx mori, L.)*

Late in July, 1884, I heard from Professor Burrill, of the State Industrial University, that a lot of silkworms which were being reared under his direction for experimental purposes were dying rapidly from an apparently contagious disease resembling the *flacherie* of the old world, and wishing to improve the opportunity thus afforded to determine the possibility of conveying this affection to our native Lepidoptera, I had, July 30, some of the dead and dying larvæ sent me by mail from Champaign. From our correspondence at the time and from an account of the experiment by Prof. Burrill, published in the Twelfth Report of the Board of Trustees of the Illinois Industrial University, 1884, we learn that the lot of worms (about 80,000 in number) in which this disease broke forth were raised from eggs derived from a perfectly healthy brood of the preceding year; that they commenced to hatch June 21; that they were kept in a clean and thoroughly ventilated building set aside for their use on the University grounds; that they began to spin July 25; that between this date and the 29th 183 cocoons were produced, but that in consequence of the outbreak of this disease among them only a single additional cocoon was made during the season. The entire remainder of the 80,000 worms perished,—commencing to die July 23, and continuing until the latter part of August.

Description of the Disease.

In a note of July 23, Prof. Burrill says of the affected larvæ that they "become yellow, shorten up; the skins become very tender so that they can hardly be picked up without bursting; body flaccid; the blood loses its clearness and becomes thick with a dirty yellowish color." Again, July 26, he writes: "They first refuse food and uneasily creep around, then become yellowish and flabby."

In the article above cited, Prof. Burrill distinguishes two forms of disease among the larvæ, as follows:

"In one case the affected larvæ became restless, ceased eating, the skin assumed a decidedly yellowish tint and ulti-

mately became very tender and easily ruptured, while the blood, unusually copious, was thin and yellow instead of its normal limpid or grayish color. Other larvæ became sluggish, continued to eat, but consumed only a small quantity of food, the body gradually became flaccid, the skin wrinkled and tough, and the color a grayish or leaden tint, and finally nearly black. These, hours or even one or two days before their death, adhered by their prolegs, or some of them, to a support, and remained quiet, at length only showing signs of vitality when touched, and at last dying while still firmly anchored to the limb or other object upon which they rested. After, and for some time before, death, the flaccid body hung directly downward from the point of attachment. If this latter happened to be near the middle of the body, the two ends hung down, the parts nearly parallel with each other. From these dead and blackened worms a decided and characteristic odor of putrescence was perceptible, tainting, when numerous, the air of the well-ventilated room."

The first of these diseases was also characterized in the Statistical Record of the State Board of Agriculture for August, 1885, by Mr. Woodworth, who conducted the experiment for Prof. Burrill. "This disease," he says, "does not make its appearance until the worms are about ready to spin, that is, near the end of the last age. The body of the affected worms assumes a somewhat granular, yellow color, instead of the natural, bright semi-transparent hue. This change of color also differs from the normal change, in that the yellow is first on the middle of the segment instead of at the ends. The skin becomes soft and tender, breaking at the least fall, and allowing the yellow body fluid to escape more readily than wounds of equal size would in healthy worms. The affected worms become very restless, crawling about and shrinking in size from loss of blood until they finally die. A few spins cocoons, which are generally soft, often bright orange, and sometimes so thin that the pupa or dead worm may be seen within. Some of the worms even pupate without spinning, and from these pupæ moths may emerge, which will sometimes deposit their eggs. When a brood of worms is attacked by this disease generally very few survive."

Several lots of the larvæ were sent me in July and August, representing both the above-described affections, the difference between which was easily discernible. The former disease was apparently that known to the French as *jaunes* (sometimes called jaundice by the English writers and by some considered the same as *grasserie)*, and the latter was unquestionably *flacherie* or *morts-flats* of the French — the *schlaffsucht* of the Germans.

The yellow color of the "jaundiced" worms was evidently due to the tint of the blood, and this, again, was as clearly derived from the great numbers of peculiar cellular bodies with which the blood was always loaded, these originating chiefly, if not wholly, in the fatty bodies, as a result of that form of degeneration of those organs in the larva which attends pupation. These bodies, when entire, consisted usually of masses of spheres, each 4 μ or 5 μ in diameter, the aggregate attaining a diameter of 30 μ — 40 μ. The individual spheres often presented a slightly angular outline, as if modified by mutual pressure, and they took no aniline color with which I tried to stain them. These bodies are evidently the mulberry cells and granules of Viallanes, as described in his admirable memoir on the histolysis of insects.* That they originated chiefly in the fatty bodies, I demonstrated by finding masses of them in portions of the fatty bodies themselves and by determining the substantially unaltered condition of all the other tissues of the affected silkworms.

In the blood of these larvæ no bacteria were found, as a general rule, although Professor Burrill occasionally recognized a Bacillus in it ; but in the alimentary canal I never failed to discover great numbers of micrococci and often also numerous examples of Bacterium and Bacillus.† These bacterial forms

* Ann. Sci. Nat., Zool., xiv. 1,—Art. 1. August, 1882.

† A transverse section of a jaundiced larva mounted in balsam without staining, shows great numbers of spherical micrococci, somewhat unevenly distributed throughout the entire thickness of the wall of the intestine, and fully as abundant in the outer portion of this wall as within. The same micrococci occur in the perivisceral spaces, being accumulated especially upon the free surface of the organs contained therein. A very few are apparent also in the sections of the fatty bodies, and occasionally in the muscles, but none occur in the skin

were not different from those observed in cases of undoubted *flacherie*, but they were usually far less numerous,—a fact which has suggested to me the following theoretical explanation of the supposed jaundice of the silkworms at the University. Assuming that the mortality was originally caused by the intestinal bacteria, we may suppose that this infection was not sufficiently overwhelming to destroy life by direct action, as seems to be the case in *flacherie*, but that it nevertheless had the effect to so disturb the balance of physiological functions as to retard the development and preparation for pupation of some of the organs, while the fatty bodies, being special stores of material accumulated for use in pupation, and so less promptly and easily affected by causes attacking the general health of the larva, went on to pupation and experienced the histolysis characteristic of that phenomenon. In other words, we may suppose, quite consistently with all the facts, that a relatively slight bacterial attack took *uneven* effect on the various parts of the animal and not immediately destructive effect on any ; that it retarded the preparations for pupation of the great vital organs, but that the fatty bodies, as if unaware of this fact, continued their course of maturation and histolysis, reaching a condition of pupal disorganization before pupation had actually occurred.

The condition of the fatty bodies of the larva affected by the supposed jaundice is well illustrated by slide 4732 of our collections, containing portions of the fatty bodies of larvæ received from Prof. Burrill on the 30th July. The cells of these organs, when examined under a power of 500 diameters, were found, nearly all of them, to have undergone a remarkable change. The contents of a few still remained minutely granular, a large nucleus being also occasionally visible, but the con-

or in the silk tubes. These micrococci are very distinctly visible, shining with a reddish light when slightly out of focus, not being rendered transparent by the mounting medium as are the tissues of the larva. They are arranged in patches and strings, the former of irregular shape, the latter sometimes containing as many as eight or ten spherules. The fatty bodies of this larva are almost solid masses of mulberry granules. The Malpighian tubules of another specimen show also, besides their normal crystalline contents, great numbers of these mulberry granules, formed within the cells or derived from outside sources.

tents of the greater number had been converted into very distinct pale granules, varying in size in the different cells from 2 μ in diameter to 4 μ or 5 μ. About 20 or 25 of the larger size were usually contained in a single cell, and a multitude—too numerous to count—of the smaller ones. Here and there in the area of the object were large irregular lacunæ evidently filled with liquid fat, as shown by the slightly crystalline character of their contents.

Whatever we may assume with respect to the bacteria infesting these worms as a cause of the premature pupal degeneration, I do not know that we have any reason to suppose that they are the only possible cause of such a catastrophe to the insect. Other influences tending to disturb seriously the balance of functions at the critical period when larval life is about to terminate in pupation might not impossibly have the same effect.

Additional details respecting this peculiar catastrophe to maturing larvæ will be given further on, under the head of *Mamestra picta*.

The Characteristic Bacteria.

As an illustration of some of the conditions characteristic of this disease, I give descriptions of well-mounted slides prepared from the fluids of one of the larvæ received from Prof. Burrill on the 30th July. The larva was dead when examined, but perfectly fresh. In the blood I found only the mulberry granules, some free and others still enclosed in their mother cells, as already described, together with blood corpuscles in various stages of degeneration. My notes at the time and a recent examination of carefully prepared slides show that no bacteria occurred in the blood.

In slide 4603, material for which was obtained by touching a cover glass to the cut end of a divided worm, I find great numbers of the mulberry granules, varying in size from 2.5 μ or 3 μ to 6 μ, the more usual diameter being, however, 4.5 μ to 5 μ. With these occurred, everywhere, myriads of micrococci, probably one fifth of the area of the field of the microscope being occupied by them where the film is of moderate thickness. These micrococci vary in form from exact spheres,

usually in doubles, to broad ovals, with the transverse diameter about three fourths the longitudinal, these likewise usually in doubles. Occasionally pairs of doubles are joined end to end in four's, but longer chains than these were not observed. The micrococci frequently occurred upon the slide in patches of fifty to one hundred, in which most of the individuals were seemingly single. The ovals above mentioned have the same transverse diameter as the spheres, differing only in length. This diameter varies but little from .75 μ, although slightly smaller singles are not infrequently found. Many of these small, as well as larger, singles are scattered separately through the field. Besides the ovals above described, occasional ovals larger than these are seen, closely resembling, in fact, *Bacterium termo*, and probably to be considered as belonging to that genus. These are about 1.5 μ in length (doubles 3 μ) by 1 μ in transverse diameter.

In the thicker part of the film very considerable numbers of excessively minute spherules were discernible, deeply stained, .5 μ in diameter, apparently identical with those described under *Pieris rapæ*, on a preceding page*, and clearly the same as those appearing in the culture described on page 286.

The slide from which the above description is taken was deeply stained with methyl violet July 30, and mounted in dammar.

Another slide, 4612, derived from the same lot of worms and similarly treated, differs only in the fact that the micrococci average somewhat smaller; that nearly every one is almost strictly spherical; and that an occasional small Bacillus occurs, 2 μ to 3 μ in length by about .66 μ in width. The ends are broadly rounded, the sides parallel, except in the shorter specimens where they are slightly convex. These bacilli are sometimes single, more commonly attached endwise in pairs. The smaller oval forms, possibly distinct, frequently show a pale center with ends heavily stained.† In this slide are a considerable

*Is it perhaps possible that the silkworm affection had its exciting cause in the disease of the cabbage worm, which made its first appearance in this region the year before?

† To this form a peculiar interest attaches in some of my other studies, reported on a later page.

number of large, regularly elliptical bodies, about 5 μ in length by 3 μ in transverse diameter. As they do not stain, they are probably crystalline, especially as it is well known that larvæ about to moult or pupate often have the blood loaded with crystals of uric acid of which the form is often not different from that here noted.

As characteristic of the second form of disease, *flacherie*, that distinguishable in the living larvæ by the pale color of the surface as compared with the lemon-yellow of *jaunes*, I have selected slide 4727, derived from the fluids of a freshly dead larva. In the blood of this specimen no bacteria were discernible, but in this slide, prepared from the mingled blood and alimentary fluids, they occur in innumerable myriads. The slides are, however, instantly distinguishable from those derived from the yellow-skinned larvæ, by the complete absence of the mulberry granules. The bacteria from the selected slide are not by any means so uniform as those in the one previously described, but vary from perfectly spherical micrococci to ovals, double ovals, and elongate bacillar rods. The spherical and oval forms of micrococci are, however, the predominant bacteria. The spheres in this slide are commonly wider than the ovals, measuring about .75 μ, while the smaller ovals are not more than .5 μ in their shorter diameter. The spheres vary in arrangement from singles to chains of considerable length, but the latter aggregates may be due to an accidental running together in the drying film. The bacilli are not distinguishably different from those described for the other form of disease. Besides the above, occasional larger broad ovals appear, similar to those doubtfully determined above as *Bacillus intrapallens*. Judging, in short, from this representative slide, one would say that the bacteria of *flacherie* of the silkworm consist of a varied mixture of round and oval micrococci of different sizes, of species of bacteria, and of small bacilli. Some of them, however, may have been of *post mortem* origin. The slide in question is beautifully stained with methyl violet, and mounted in dammar.

Contagious Character of the Diseases.

I had no opportunity to observe the progress of these diseases in the silkworm, but Professor Burrill was entirely confident of their contagious character as exhibited under his observation. On this point he says* : "That the worms came from good eggs, and were, for a considerable time, perfectly healthy and wholly free from the malady which finally overtook them, we have the best of evidence. The disease which carried them off was not hereditary. It was not lurking unobserved during the more favorable weather in the living or dying worms. Its introduction occurred about, and probably at, the time of the first heavy rains spoken of, but we confidently know that it could have been artificially introduced without the rains or the wet weather at all. Moreover, the worms continued to die after the weather cleared up, and after every precaution had been taken to put them under the best possible conditions. We constructed new racks in a room not previously used, picked out the healthiest worms and moved them to the new and clean quarters, where, afterward, the temperature and other conditions were as favorable as could be desired ; but the ravages of the disease continued with no perceptible abatement. To further test the matter, other apparently healthy worms, voracious feeders, growing rapidly, were put out upon the open hedge, where they were watched from daylight until dark to keep off the birds, and where, for a time, they seemed to thrive under the favorable skies and wide isolation ; but here, too, they gradually fell victims to the destroyer. In each of these places about five hundred worms were placed, from which, as was before said, one cocoon only was secured, and this from the out-of-door lot. The latter did live longer than any of the others, but at length as surely succumbed. Another experiment proved equally futile ; viz. that of spraying the food with an aqueous solution of carbolic acid. No apparent improvement followed this treatment.

It may be said that our disaster followed in consequence of retarding too long the hatching of the eggs by keeping them in

* Twelfth Rep. of the Board of Trustees of the Illinois Industrial University, pp. 90, 91.

an ice-house, thus pushing the feeding season out of the natural time and subjecting the worms to unfavorable summer heat, or providing them with leaves too far advanced towards maturity. This might, indeed, seem plausible had not several other lots, fed in the vicinity, but not so retarded, died in the same way. It is interesting to note that in some of these small and isolated experiments in silkworm feeding, certain lots from the same kind of eggs as our own, produced from the same lot of moths, fed on the same kind of food, remained perfectly healthy and produced good cocoons, while others totally failed. It seemed that in every case where what appeared to be the disease called in this paper *flacherie* became once introduced, few or none of the worms lived to spin passably good cocoons. Most of them died after the third or fourth moults, and after, therefore, no little care had been bestowed upon them."

My own observations on this phase of the subject were of an experimental character, and will be found in detail under the head of Experiments for Artificial Infection. Here I need only say that they demonstrated the possibility of affecting with disease healthy larvæ of the common cabbage butterfly *(Pieris rapæ)* by means of artificial cultures of the bacteria occurring in the sick silkworms,—these cultures being made in beef broth and applied to the cabbage worms in confinement by sprinkling or spraying their food.

Artificial Cultures.

Our first cultures of the bacteria of the silkworm were made July 30, in test tubes of beef broth, by the methods described above, in my account of the cabbage worm disease, the material for infection having been obtained from a yellow-skinned larva (affected by jaundice) received on the same date from Professor Burrill, of Champaign. The larva used was recently dead, but still perfectly fresh. Two cultures were made, one from the blood and one from the alimentary fluids. No bacteria were discernible in the blood, either in fresh preparations or in mounted films, the latter presenting only numerous and excellent examples of the mulberry cells and granules characteristic of the disease. The slides prepared from the fluids of the alimentary canal, however, exhibit numerous

specimens of a strictly spherical Micrococcus, occurring usually in doubles, measuring 1 μ in diameter, with an occasional oval example apparently elongating for division, and then about 1.5 μ in length. These micrococci stained readily with methyl violet.

In the test tube infected from the blood, curiously enough this Micrococcus reappeared in a perfectly pure culture. The fluid, infected July 30, was seen to be milky on the 1st of August, and many micrococci were visible in doubles and chains, the latter being unusually abundant. On the prepared slides, less heavily stained than the originals from the silkworm, these micrococci measured a little less than those of the alimentary canal, the diameter usually falling between .75 μ and 1 μ, rarely attaining the latter dimension. Chains of six or eight were not uncommon.

The culture derived from the alimentary canal of this larva was unexpectedly impure and not altogether comprehensible. The fluid was observed to be milky August 1, and many micrococci appeared in fresh slides, both in doubles and chains. A perfect film, distinctly stained, but rather pale, shows, however, a variety of forms. Most conspicuous, but not the most abundant, are doubles and short chains of three to six of a strictly spherical Micrococcus, deeply stained, entirely similar to those above described, but averaging smaller, their mean diameter being a scant .75 μ. Besides these are short, broad ovals, a little less deeply colored than the above, of the same transverse diameter, but a fair 1 μ in length, some, indeed, falling scarcely short of 1.25 μ. In addition to these and of the same transverse diameter, we see, rarely, rod-like forms, apparently bacilli, measuring from 3 μ to 4 μ in length; and, finally, thickly scattered, everywhere more abundant than any oval form, are very minute spherules, always in singles (except in now and then an instance seemingly accidental), measuring a scant .5 μ in diameter. These are well stained and conspicuous, and unquestionably do not belong to the film. They are extremely like the smaller form of cabbage-worm micrococci which I have already described. Their appearance under the circumstances suggests the possibility of their being bacillar spores, but the bacilli in the film are far too few to permit this

explanation ; nor did any of those noticed seem to be spore-bearing. The impurity of this culture makes the supposition plausible that some of the bacteria of the orignal infection were introduced by accident and not derived from the silk-worm. The check tube, however, remained unaltered, as usual; and it seems to me more likely that the originals of all these forms were really derived from the alimentary canal. It is not to be supposed that the alimentary contents of a larva long diseased, and, indeed, actually dead, should remain wholly free from invasion by bacteria other than those strictly characteristic of its disease.

The cultivation of bacteria from the blood, although none were microscopically demonstrable in the latter itself, seems to me not a remarkable phenomenon (especially as the fluid was derived from a dead larva), since it could scarcely be credible that the circulatory fluids should, under such circumstances, be entirely free from the peculiar germs of the disease to which the larva had succumbed. It must be remembered that a single individual Micrococcus would be sufficient to start the culture in the tube, and that the quantity introduced into the beef broth was much greater than that represented by the films microscopically examined. Furthermore, an occasional Micrococcus in a stained film may readily be overlooked or passed as doubtful, since the difficulty of distinguishing single individuals from accidental granulations of the film itself forbids positive identification of the micrococci unless they occur in numbers sufficient to make their character unmistakable.

Another culture, commenced July 30, from the silkworm 4603, the bacteria from which were described under this number on page 281, was examined August 1, at which time the fluid was observed to be milky and found swarming with micrococci and a few examples of Bacterium (?). (The latter, it will be remembered, were also observed in the original material.) The resultant culture was possibly impure, the two forms appearing on the slides being distinguished, however, only by the positive strong stain of one and the very delicate stain of the other, shapes and sizes not being appreciably different.* That distinctly

*Those lightly stained were probably the empty walls of dead examples.

stained unquestionably agreed in every particular with the common spherical Micrococcus of the original silkworm material, except that it measured a trifle smaller, scarcely averaging 1 μ, although many individuals and doubles were fully that size. This culture was preserved for experiment and used as an infection fluid on the 9th August. The results of this attempted infection will appear under another head.

Still another culture, commenced and examined upon the same dates, yielded an abundance of the spherical Micrococcus most frequently mentioned above, together with occasional examples of a Bacillus 3 μ or 4 μ in length and about 1 μ in transverse diameter. These last were, however, too rare to have any special significance, except as a slight adulteration of the culture.

The next culture attempted, commenced July 31 and examined August 4, is of especial interest, as it resulted in the complete displacement of the normal Micrococcus of the silkworm by another organism present in its fluids (the questionable *Bacillus intrapallens* already mentioned*), but in small numbers.

This culture was made from a silkworm of the original lot received from Professor Burrill, July 30, the beef infusion being infected from a dead worm. The fluids of this larva contained vast numbers of the ordinary silkworm Micrococcus, somewhat under the usual size, averaging, indeed, only about .75 μ. An occasional large Bacillus, 4.5 μ long and 1 μ wide, also occurs on the slides made from this individual. Besides the above is the organism already mentioned, varying in form from a broad oval to a Bacillus-like rod, characterized by a pale center staining little or none, and heavily stained extremities. The culture examined August 4 contained vast numbers of this organism and apparently nothing else. Most of those appearing in the films from this culture were much smaller than the original, all the stages, in fact, appearing, from a simple sphere scarcely, if at all, distinguishable from a Micrococcus, to the

* This organism displaced similar cultures made from the larva of *Datana angusi* presently to be reported on, was preserved through the winter, cultivated the following season, and then applied effectively to the destruction of larvæ of other species.

rod-like form or double elongate oval, the paler centers commencing to appear in the oval and becoming more conspicuous as this elongates.

A single somewhat later culture, commenced August 4, did not differ materially in results from those preceding. No bacteria were discoverable in the blood of the larva, used by prolonged and careful search, but the alimentary fluid contained the usual Micrococcus. Five days later the infected infusion was decidedly turbid, but without either film or sediment. Besides an occasional short Bacillus in active movement, it contained only the spherical Micrococcus of the usual size.

The slides of these various cultures clearly demonstrate the presence of a spherical Micrococcus, varying in diameter from .75 μ to 1 μ, as the characteristic Bacterium of the disease from which these silkworms were perishing, and likewise the practicability of artificially cultivating this Micrococcus in neutralized beef broth by infections from the alimentary canal and from the blood. Although the Micrococcus itself was not demonstrable in the blood by the microscope, it was obtained therefrom by cultures in which it appeared without admixture of other forms. Intestinal cultures were, however, liable to contamination by other bacteria but doubtfully connected with the disease, among which was the form last described.

Infection Experiments.

I found it by no means easy to provide means for testing satisfactorily the possibility of conveying the disease of the silkworm above described to other demonstrably healthy insects. The late period of the occurrence of the disease under my observation made it impossible to use other lots of the silkworm itself in the experiment, and no other lepidopterous larva was sufficiently abundant at the time, except the cabbage worm. This, however, had alread been found, the previous year, to suffer extensively from an extremely destructive disease of its own, and although at the time the experimental stage of my studies of the sick silkworms had been reached, no evidence of disease among the cabbage worms had yet appeared in the fields, I had every reason to anticipate its outbreak among them,—a fact which made me very doubtful of really bringing

the matter to a decisive test on that species. The occurrence of a spontaneous outbreak of the common cabbage worm *flacherie* among the lot under experiment, would of course arrest the progress of the experiment, and might even so mask the result as to mislead.

This accident, in fact, occurred to my first two experiments, begun August 9 and 10. Not only did the cabbage worm affection appear in both the experimental breeding cages and the checks, but the latter lot as well as the former gave evidence of infection from our silkworm material. The latter fact convinced me that my arrangements were inadequate for the protection of my check lots against accidental infection with the experimental material. These lots were placed at a distance from those purposely exposed to disease, but in another part of the same large hall, and were attended by the same assistant. In previous experiments, not yet detailed, with other larvæ, I had already had evidence of slight unintentional infection of the check lots by this too close association with those under treatment, and now arranged another experiment on a wholly different plan.

Careful examination was made of all the cabbage fields near Normal, and one was selected which showed no trace of the proper disease of the cabbage worm. From this field two lots of caterpillars were selected, twenty-five in each, those for experiment by the assistant whose duty it was to make the infections, and the check lot, by an intelligent student of the Normal school, who did not visit my zoölogical laboratory at all. The first lot was brought to the office and placed in a clean and disinfected cage in the usual place, but the second or check lot was taken by the student mentioned directly to his own home and confined in a new breeding cage. Care was taken that both lots should be fed and treated alike, except for the infection, but no opportunity was given for any communication between them. The results in this case were more satisfactory, and confirmed my suspicion that our check lots had not before been sufficiently isolated.

History of the Infected Lot.—The food of the twenty-five cabbage worms selected especially for experiment, was sprayed on the 6th September with beef broth infected nearly a month pre-

viously from the fluids of a silkworm recently dead from jaundice. Unfortunately, from some oversight, neither slides nor detailed notes were made of this culture until the experiment upon the cabbage worms was instituted. The beef broth, nearly a gallon in quantity, contained in a large receiver, the tube of which was closed with a sterilized cotton plug nearly six inches in length, had promptly become turbid, as usual, and was soon opaque with bacteria. By the 6th September the development of the bacteria had apparently nearly ceased, a thick deposit covering the bottom of the jar. The fluids at this time contained vast numbers of spherical micrococci .7 μ to .8 μ in diameter, mostly in doubles, apparently identical with those occurring in the silkworm. The culture, however, which had been several times opened for examination, was not at this time wholly pure, but contained likewise bacteria and large and small bacilli. These occurred, however, in relatively insignificant numbers, and the fluids when poured out presented no odor of putrefaction, but had, on the contrary, only the faint indescribable smell characteristic of the cultures of all our insect bacteria.

After infection on September 6. the cabbage worms were fed with fresh food collected for them daily. Their cage was kept in a large room, before an open south window, was thoroughly cleaned each day, the paper covering the floor of the cage being removed and burned, all the litter and *débris* destroyed, and the larvæ carefully transferred to fresh food upon clean paper.

A single individual died September 8, evidently from accidental injury. Three of the larvæ pupated on the 10th. On the 11th two died, apparently of disease. The fluids of these were carefully examined and found to swarm with micrococci. Of these covers were prepared in the usual form. The first slide, made from the blood, contains large spherical micrococci, nearly all in doubles, 1 μ in diameter, excellently stained with violet. The bacteria of the second slide, representing the contents of the alimentary canal, were more various in form. In addition to the above large Micrococcus, 1 μ in diameter, many slightly double ovals of about the same transverse diameter occurred, together with several .7 μ wide, most

commonly arranged in small groups; occasionally, also, an unsegmented rod, possibly Bacillus. Nothing representing the minute spherical micrococci characteristic of the native disease of the cabbage worm occurred in this specimen. The next day, September 12, one larva pupated and four perished. The first of these examined was already blackened and deliquescent. It contained nothing but large and small micrococci strictly spherical in form, the large one 1 μ in diameter, the other about .6 or .7 μ. Both occurred usually in doubles, but not unfrequently in singles or short chains. Both stained well in methyl violet, and good slides were prepared. The smaller form of the above micrococci was found only in the blood, and the larger only in the intestine, as indicated by the stained slides from these two sources.

The second larva studied was soft and grayish green, but the skin was tougher than usual, and showed little tendency to the characteristic deliquescence of the cabbage worm disease. The fluids were yellowish white, and contained great numbers of large and small spherical micrococci, the larger 1 μ in diameter, the smaller .6 or .7 μ.

The third specimen, smaller than the preceding one, was a little darker in color, the fluids yellowish green and containing identical micrococci. Both forms were spherical and of the same dimensions as those just described. A single Bacillus was also noted, 2.5 μ in length, and an occasional double oval occurs upon the slides (probably Bacterium) each oval element about .8 μ long.

The fourth specimen was flaccid, but bright green, its fluids thick and milky white. It contained a moderate number of large spherical micrococci, identical in appearance with those described above, varying in character from .8 μ to 1 μ. Besides these, the blood was literally loaded with large spheres, evidently mulberry granules, occurring singly and in masses, the diameter varying from 2 to 4 μ. A close correspondence in the condition of this larva to that of the silkworm affected with jaundice will at once be noted.

Four other larvæ, two of which died September 13 and two on the day following, were briefly examined, but not carefully studied. Their fluids presented no considerable differences from

those already treated. On the 15th another larva pupated, and a second died during the night which had been reported sluggish the previous day. The body was shrunken, not very soft, a little brown, but the general color was still the usual green. The fluids of the specimen were very white and thick, and contained vast numbers of mulberry granules, both singly and in clusters, together with great quantities of oval micrococci (some in chaplets of four) and occasional individuals of Bacterium, some of the latter in actual motion. The mulberry granules were strictly spherical, and varied in size from 1.5 μ to 3 μ in diameter.

Another larva which died was originally paler than natural, but not white. Before examination it had blackened and turned very soft, but was not deliquescent. Slides prepared from it contained *débris* of tissues, muscular and other, and vast numbers of minute spherical micrococci from .5 μ to .7 μ in diameter. No flagellar motion was detected in the fresh slides, and no other forms are apparent in the stained mounts.

Another example, small and shrunken, a little discolored, dried up in a few hours, and became hard and brittle. It was not especially studied. On the 17th of the month the last remaining larva died. It was not discolored, and I could find no bacteria in the blood or other fluids. The cause of its death, in fact, was not apparent. At this date a blackened pupa from the cage, evidently not long dead, was found full of a blackish fluid, which contained vast numbers of a small spherical Micrococcus (.6 μ in average diameter, commonly in doubles) and nothing else, except occasional mulberry granules 2 μ in average diameter. Of the individuals which pupated, six emerged successfully, three were deformed, and two failed to complete their transformations.

History of the Check Lot.—This lot, placed in a new breeding cage September 10 with fresh cabbage, was kept under continued observation until the 28th. One of the specimens died the first day from an accidental injury; one pupated on the 12th; and two others were necessarily crushed in opening the cage, having commenced to pupate on its sliding glass front. On the 14th four examples pupated, and two more upon the 15th, at which time fifteen healthy larvæ remained. The more

rapid pupation of these specimens will be noticed, as compared with those treated with the infection material,—a fact consistent with what I have uniformly observed with regard to the effect of these diseases.

On the 17th four worms were drowned in a dish of water containing the food plant in the breeding cage. The fluids of these worms were carefully examined with a microscope, and careful studies were made of stained covers of their blood and alimentary contents, but no possible bacteria of any sort were detected in them. On the 21st three more larva pupated, and on the 23d three died. Unfortunately, the latter fact was not reported by the assistant in charge in time to permit an examination of these dead worms. All the remaining larvæ pupated, the imagos commencing to emerge on the 26th.

Although the results of the foregoing experiments were somewhat less definite than might be desired, yet they clearly indicate the transference of the disease affecting the silkworm to healthy larvæ of *Pieris rapæ*. It would perhaps have been difficult to establish by a study of the bacteria alone any marked difference between the disease resulting from this experiment and that native to the cabbage worm, but the symptoms of the two diseases were so unlike as to make it impossible to confound them. The general absence of the peculiar discoloration of the common *flacherie* of the cabbage worm, and of that rapid *post mortem* deliquescence even more characteristic of it, leave no doubt as to the actual difference between this induced disease and the spontaneous affection. That the artificial disease was identical with that of the silkworm, differing only in such a degree as was to be expected when attacking such widely different larvæ, is rendered probable, not only by all the attending circumstances, but also by the occurrence in the cabbage worm of the myriads of mulberry granules characteristic of the affection in the silkworm. This fact is especially significant, since in all our numerous examinations of the native *flacherie* of the cabbage worm this condition of the fluids was not once observed.

I followed this experiment with a similar one in the field, applying the same fluid to a number of cabbages infected by

the worms and selecting others as a check on those treated, but the appearance in this field, at about this time, of the common *flacherie* of the cabbage worm, and the death, from this cause, of several of both lots of larvæ interrupted the experiment. The general outbreak, also, of the same spontaneous affection of the Pieris larvæ elsewhere in the vicinity, precluded all attempts at a repetition of these field experiments.

THE YELLOW-NECKED APPLE CATERPILLAR.

(Datana ministra, Drury.)

On this species my first studies of the bacterial diseases of caterpillars were made in the autumn of 1883. The affection which attracted my attention broke out in our breeding room shortly after the larvæ were collected, but was not seen among the species anywhere in the field. It probably was not different from the disease well known to entomologists who rear caterpillars to the imago, especially liable to appear in close and sultry weather, and when the breeding cages are insufficiently ventilated.

A lot of the larvæ, two or three hundred in number, obtained July 23, was reported to me, August 1, to have been mysteriously dying for several days at the rate of two or three a day. The small room in which they were kept was open to the south by a large window, and breeding cages of ample size were used, so placed as to be well ventilated. The larvæ were fed and the cages cleaned daily.

Description of the Disease.

Except that no change of color was usually perceptible, the symptoms of this disease were not especially different from those which have been already given for the silkworm and cabbage worm. Sluggishness and evident weakness and loss of appetite were the first noticeable phenomena. A larva while resting upon a vertical surface would often partly lose its hold, and hang only by a few of the legs,—this occurring long before the power of active locomotion was lost. As a very common thing a discharge of a brownish fluid from the vent occurred early in the disease, but occasionally this symptom was not

observed. As a consequence of this purging, the body would become soft and flaccid and somewhat shrunken,—an appearance not presented by those in which the purging did not occur. Occasionally some portion of the body, usually the central or posterior part, became darker before death, but much more commonly the larva retained its natural hue. The approach of death was gradual, the affected insect becoming more and more sluggish and insensible to irritation. *Post mortem* changes were neither so rapid nor so extreme as in the cabbage worm, owing probably, in part, to the thicker and tougher skin.

The fluids escaping from the vent were microscopically examined, and found always swarming with bacteria,—many of them not infrequently having the flagellate motion of Bacterium proper, but the greater number of them being clearly Micrococcus. If a droplet of the blood were obtained before death, it rarely gave any evidence of bacterial affection, the only cases in which this was seen being those in which an *ante-mortem* blackening of the body was observed. After death, however, the blood invariably swarmed with the same bacterial forms which were found earlier in the intestine, the ordinary septic species soon developing rapidly. The alimentary canal usually contained, both before and after death, vast numbers of Micrococcus, and also, not infrequently, true Bacterium, but bacilli or other bacterial forms were rarely found. The micrococci occurring were not by any means as uniform as in the cabbage worm and silkworm, both spherical and oval species of various sizes often appearing on the same slides. The intestine was commonly filled with food little, if at all, digested. In only one instance was the alimentary canal empty and partly filled with gas.

The Characteristic Bacteria.

The bacteria which, from their abundance and uniform presence, must be regarded as characteristic of this affection, occurring as they did in the still living larvæ almost to the exclusion of other forms, were oval and spherical micrococci,—sometimes one, sometimes the other, and sometimes both commingled in variable proportions. The oval micrococci were

usually in singles and doubles, the spherical ones commonly in doubles and short chains of four to six ; in the latter case, often taking on a quadrate form. The ovals varied in length from 1 μ to 1.4 μ, and in transverse diameter from .8 μ to 1 μ. The spherical and quadrate forms were nearly always under 1 μ in diameter, usually averaging about .8 μ. Both forms stained readily with both methyl violet and brown, and occurred frequently in patches or colonies in the intestinal canal.

I mention here a point of especial interest in relation to subsequent attempts at culture and infection. I studied on the morning of the 5th August the fluids of a larva which had died during the night. The blood obtained by snipping a proleg was thick and gray with bacteria, as were also the intestinal fluids, many in both blood and alimentary canal having the form and flagellate movement of Bacterium. Occasionally a string of four, attached end to end, would be seen in serpentine movement across the field. Well-stained and permanently mounted slides of their fluids show three bacterial forms: one large oval, undoubtedly *Bacterium termo;* one a smaller oval (the Micrococcus already described); and the third a somewhat peculiar oval form which might be understood as a single oval 1.5 μ long, with a pale center, or as a short double oval whose division was indicated, not by indentations of its margins, but by a thinning of its central part. The study of slides subsequently made under other circumstances enables me to say that this form last mentioned is really a developing Bacillus of a peculiar character which, matured, is short, broad, and quadrate, its central portion pale when stained, and the ends contrasting by a positively darker tint. Unable to identify this form with anything described, or to obtain through my botanical friends any specific determination of it, I shall refer to it in this paper, merely for convenience sake, under the provisional name of *Bacillus intrapallens.**

*I do not know that this is a distinct species, or intend so to imply. *Bacillus subtilis* sometimes presents the peculiar segregation of its contents here described, under what peculiarities of circumstance I do not know, but never, as far as I have observed or can learn, until the full size of the cell has been reached. In the above Bacillus, on the other hand, it was usually evident as soon as the young cell was large enough to show it.

Contagious Character of the Disease.

I made no effort to determine experimentally the question of the contagious character of this disease in *Datana ministra*, and can only report that it gradually invaded all the breeding cages of this and an allied species, *Datana angusi*, which we found during the season. Many of these were kept at a distance from those suffering from the disease, either as reserve or check lots, with the hope of protecting them from its operations ; but as they were, at farthest, in adjacent rooms, and as we passed freely from one to the other, none of them can be said to have been *isolated.* The bacteria appearing in the walnut Datana *(D. angusi)* were not different from those infesting the other species, except that in our observations the spherical form was usually the characteristic one for this species. Still, both spherical and oval micrococci were noted in a multitude of instances.

Artificial Cultures.

Our first culture illustrating this disease was commenced September 6, 1883, with material obtained from an example of *Datana angusi* seriously affected, but not yet dead. The slide made from the fluids of this larva is not by any means pure. It shows in nearly equal quantities the spherical and oval micrococci described in the preceding section, the oval form mostly in doubles, each pair varying from 2.5 μ to 3 μ in length, and being .75 μ in transverse diameter. The spherules were mostly in doubles (the pairs somewhat under 2 μ in length) and in chains of four or more, the elements of which were sometimes quadrate. Many of both ovals and spheres were aggregated in large, dense patches. Very rarely, also, occurred a larger form, not measured, apparently a Bacterium.

Sterilized and neutralized infusion of beef was infected with fluids from this larva, by the methods and with all the precautions already described. This infusion speedily became milky, and slides made a few days after the culture was begun show clearly a reproduction of the spherical Micrococcus of the original fluid, but of no other form. In size, general appearance, and reaction to staining fluids, this differed in no par-

ticular from the original. Singles occurred occasionally, but most of the specimens were in doubles, no chains being noticed.

Additional slides, mounted October 2, show likewise the same spherical Micrococcus without admixture, or change in size or mode of aggregation ; and still another series mounted from the same tubes, April 9, 1884, represent a still pure culture of what was probably this same Micrococcus. The specimens differ only by the somewhat smaller size, rarely surpassing .8 μ, — a difference probably to be accounted for by an exhaustion of the nutritive fluids, certain to have occurred during the seven months which had elapsed since the culture was begun. It should be said, also, that the slides of this last stage are less distinctly stained than the preceding, the micrococci very probably being dead.

After a careful re-examination of these materials I do not doubt that this was a successful culture of the spherical Micrococcus, preserved through the winter, practically unaltered, in a test tube plugged with cotton. It should be added that the check tube remained throughout unchanged.

An interesting culture was begun September 8, the material being obtained from a larva of *Datana ministra* dead several hours. The slides representing this larva are impure, the fluids from the alimentary canal containing not only spherical micrococci, but also a few ovals, and great numbers of bacilli. The spherical micrococci range in diameter from 1 μ to 1.25 μ, and are occasionally indistinctly quadrate, especially when occurring in chaplets (as they frequently do). A few doubles measure 3 μ. The bacilli are all slender, varying greatly in length (from 3 μ to 5 μ), but all .7 μ in transverse diameter.

The beef broth infected with this material on the 8th September was observed on the 15th to have become slightly milky, and, examined, was found to contain micrococci in couples and chaplets, chiefly arranged in the latter form. The slides made from this culture contain no bacilli, but only spherical or subquadrate micrococci in doubles and strings. These average a scant micro in diameter, some, however, reaching 1.25 μ. October 2 these fluids were found to contain only the same Micrococcus, not distinguishable in any way from

those on the slide already described ; and even on April 24 of the following year, the test tube, which had been preserved over winter, yielded only the same Micrococcus, as shown by well-stained and mounted slides prepared at that time. Magnified 1400 diameters and carefully measured, the single spherules vary from 1 μ to 1.25 μ in diameter.

From the foregoing I infer a verification of the experiment just reported, by a second successful culture of the spherical Micrococcus of the Datana larva and its preservation, uncontaminated, until the following year.

The only gelatine film cultures made with this material were begun September 8. Six films of solid beef gelatine, touched with a needle point dipped into the fluids of a larva of *Datana ministra* and inverted over a deep cell containing a droplet of distilled water to prevent drying out, exhibited September 10 a rapid growth of the infection,—each, originally a mere point, being now about the diameter of a pin head, and some having penetrated upwards the thickness of the film. The growth of this mass was in the form of thick finger-like processes, extending upwards through the gelatine film,—the marginal increase however being uniform and continuous. When warmed, these gelatine-film bacteria took on the flagellate motion of Bacillus, and the stained slides made from them strongly indicate that they are young individuals of *Bacillus intrapallens.*

Infection Experiments.

A few experiments with cultivated material were made upon other Datana larvæ obtained from time to time out of doors, these being divided into experimental and check lots, and the food of the former treated with infusions containing the cultivated bacteria. These were among our first experiments, and the control cages were evidently imperfectly isolated. As a consequence, the experiments were brought to naught by the appearance of *flacherie* in all the cages with which we had to do. In each instance, however, the mortality was more immediate, and at first much greater, among the lots treated with the bacterial cultures than among those not purposely infected ; but the results arrived at are not insisted on, and no detailed account of these experiments is deemed advisable.

THE WALNUT CATERPILLAR.
(*Datana angusi*, G. & R.)

I have to report under this species a series of observations, cultures, and experiments, the longest which I attempted. Although these failed, in part, of their original purpose, they brought out incidental and unintended results of considerable interest, and seem to me worthy of somewhat detailed description.

On the 14th of August, 1883, a lot of the larvæ of *Datana angusi* were collected from a black walnut tree (*Juglans nigra*) in the university grounds at Normal, and brought to the office for experiment. Seven of these were placed in a breeding cage in the further end of the Laboratory, somewhat removed from all the other experimental lots. On the 30th of August one of these was found dead in the cage, having certainly perished since the preceding day. The body of this individual was very limp and flaccid and considerably shrunken, and no food occurred in the alimentary canal. Mounted slides of the blood show vast numbers of the short, broad Bacillus, with rounded or subtruncate ends and pale central area, which I have distinguished as *Bacillus intrapallens*. The blood was, in fact, a nearly or quite pure culture of this organism, only some smaller and apparently undeveloped forms being possibly micrococci, but more probably the above Bacillus in its earlier stages. These bacilli measured upon an average 1.25 μ by 2.5 μ, and occurred singly and in doubles, the doubles with truncate opposed ends and broadly rounded free extremities. Besides the above, the intestinal contents presented spherical micrococci, usually single, but occasionally in process of division, .8 μ to 1 μ in diameter. I strongly suspect that these apparent micrococci also were the above *Bacillus intrapallens*, undeveloped.

The next morning a second larva of this lot was found dead, having apparently succumbed several hours previously. The intestinal fluids contained a great variety of bacteria, including Bacterium, and multitudes of minute spherical micrococci; but no slides or precise descriptions were prepared.

On September 2 another larva died which had been ailing for two or three days. But very few bacteria were found in the blood, while the intestinal fluids were full of double ovals,

not flagellate. Mounted slides show numerous spherical or slightly quadrate micrococci, with many single and double ovals. The spherical form is .75 μ to 1 μ in diameter, some of the single ovals attaining a length of 1.5 μ. The usual length of the latter is, however, about 1.25 μ.

Another larva of this lot died during the night of the 3d September, and was examined on the following morning. Its intestinal contents were brown and nearly solid, requiring to be moistened for examination. They were noted as "full of single and double micrococci," but the slide prepared is so excessively poor that nothing satisfactory can be determined from it.

From this last larva a culture was made as follows: On September 1 freshly prepared strong beef broth was filtered, while hot, through sterilized filter paper into a four-ounce flask which had just been heated for an hour in an oven at 275°-300° Fahrenheit. This was stopped at once with a three-inch plug of raw cotton, freshly sterilized by several hours' heating as above, and was boiled with the plug inserted. This flask was left undisturbed until the 4th September, when it remained perfectly clear. It was then boiled five minutes without removing the plug and left to cool. A particle of the alimentary contents of the above larva, about as large as the head of a pin, was now taken up on the point of a recently heated needle. The plug of the flask was removed, the infection material introduced, and the flask plugged again with fresh sterilized cotton still hot from the oven. A check flask was set aside at the same time.

On the 5th September the fluid was evidently turbid throughout, but especially so at the edges, and a slight film was apparent upon the surface. The plug was loosened, and a droplet of the fluid was obtained upon a freshly heated glass rod. The mounted slide of this material was, unfortunately, worthless, but, from notes made at the time, it appears that the bacteria occurring were rather large "double ovals," nearly all motionless, but with an occasional flagellate individual. Compared with the original infection material, there was no question of the identity of the two.

On the 6th September these fluids were milky, and a film had formed on the glass at the edges, where the fluid had a

somewhat ropy appearance when shaken. The check flask was perfectly clear. On September 8 the infected infusion was covered with a thick white surface-scum and the whole mass of the fluid was strongly turbid. A droplet of the liquid contents was now drawn out for examination, with a freshly-made capillary tube pushed down through the plug. The thin film upon the slide was milky with bacteria, which presented, under the microscope, an appearance of double ovals with occasional small clusters or patches of the same object, and occasional strings of three. No other form was seen among myriads passed under the eye, and no flagellate motion was detected ; this was, consequently, an unmistakably pure culture of this single organism. Admirable slides of this material, prepared at this time, further illustrate the purity of the culture, and show that many, perhaps all, of the so-called "double ovals" of my notes were immature *Bacillus intrapallens*, in most of which the pale center was but just beginning to show. On the 13th of September a number of additional slides were made from this same flask, the contents of which were now extremely turbid, the lower half thick with a whitish sediment, and the surface and the flask about the edges covered with a scum. These slides contain only the above Bacillus, somewhat increased in size, and showing the characteristic pale center more distinctly. Considering the frequency with which this form occurred in the dead Datana larvæ of this lot, I have no doubt that this was a successful culture of this particular Bacillus.

On the 17th September these fluids were selected for an experiment intended to test the possibility of preserving throughout the winter the bacteria contained in them, and a number of films were spread upon glass slides previously sterilized by heating, dried immediately with moderate warmth, and laid away for preservation. At the same time small glass tubing was taken, heated thoroughly in the flame of a lamp, and divided by melting, while still almost red hot, into short tubes closed at both ends. As soon as cooled, these partially exhausted tubes were first filled with the bacterial culture by breaking off beneath the fluid, with sterilized forceps, the tip of the tube, which then filled by atmospheric pressure; and were then immediately re-sealed by heat and laid away in

cotton for the winter. Several of them were opened in the spring and summer of 1884, at various dates, and found always to contain only a pure culture of the original Bacillus, the results of the first examination, made April 4, not differing in this respect in any particular from the last, made July 30. These bacteria stained much less freely than those in the fresh culture, — a fact probably to be accounted for by their dormant condition. Occasionally a spherical or subquadrate form, 1 μ to 1.25 μ, is distinguishable in the field by a deeper stain, — possibly a spore of the preceding.

Next came a culture in beef broth made by the usual method from the contents of these tubes on the 23d of June, 1884. Two days later this was slightly turbid, decidedly so on the 26th, and on the 27th, when slides were made and the material was used for an infection experiment, they were almost milky. The contained bacteria now consisted of two forms: that frequently mentioned above as *Bacillus intrapallens*, and a spherical form indistinguishable from rather large micrococci. The bacilli occurred singly, doubly, and in strings, were 1 μ by 3 μ in typical specimens, but varied considerably, especially in transverse diameter, reaching sometimes a width of 1.5 μ. The spherules, on the other hand, averaged about 1 μ in transverse dimension. These occurred in various arrangement, but especially in long chaplets. Many of them presented a slightly quadrate outline and in a great number of instances strings of these were continuous with shorter filaments of the bacilli. Occasionally I satisfied myself that two or three of these spherical forms were contained within the Bacillus cells; that they were probably, indeed, to be considered as spores of the cells or, as seems to me more consistent with the facts, as an alternate form of the Bacillus. They seemed not to be developed by the transformation of the contents of an entire Bacillus filament, but rather to be separated off from the end of such a filament by a transformation of the protoplasm in the thickened ends of the cells.

Numerous other cultures were made from this same material. One commenced July 30 was found, August 1, to be decidedly turbid, and on the 2d to have formed a thin transparent pellicle over the whole surface. On the 3d this tube

was opened. The fluid was covered with a rather thick film made up wholly of the above *Bacillus intrapallens*, as determined at the time and as shown by beautifully stained and well-mounted slides which I have studied recently. Many of these were in long filaments, but none showed any sign whatever of flagellate motion. This culture, like the preceding, was subsequently used for an infection experiment.

Similar cultures from the same material were made April 21 and 24, three tubes being inoculated on the latter date. From all these was obtained the same bacillar form, having occasionally associated with it the sphericals already mentioned, and in a single instance containing also a small Micrococcus about .5 μ in diameter.

The general results of these cultures unquestionably establish the possibility of preserving through several months the bacterial form here dealt with, and afterwards cultivating it successfully in beef broth.

I have next to describe the infection experiments with this Bacillus, showing the possibility of instituting disease in healthy larvæ by means of it, and of procuring its multiplication within their bodies for some days subsequent to the infection.

THE ZEBRA CATERPILLAR.

(*Mamestra picta*, Harris.)

A small colony of zebra caterpillars found on cabbage near Bloomington was brought to the Laboratory June 1, for infection experiments with one of the above cultures,—that begun June 23 and found to contain the *Bacillus intrapallens* and the spherical Micrococcus, as detailed above. A quantity of this fluid was poured into a dish June 27, and a single cabbage leaf was soaked in it for an hour and then fed to the larvæ. These ate freely of it, and were thereafter fed daily with fresh cabbage and carefully attended, this first infection, being the only one purposely made. A check lot of the same brood was placed in a separate cage, but unfortunately removed only a few yards from that infected.

On the next day a single larva of the first lot was found almost dead, and, being isolated, died during the night.

Examined June 29, at nine o'clock a. m., the fluid obtained by snipping off a proleg was found swarming with large bacilli, motionless at first, but beginning to move actively in all directions when exposed to the air under the cover. These bacilli measured from 2.5 μ to 5 μ in length, one apparently undivided reaching a length of 8 μ, with a transverse diameter of 1.5 μ. These presented no appearance of spores; the ends were broadly rounded, the sides parallel. Small numbers of micrococci occurred in the same slides, about .7 μ in diameter, strictly spherical, in singles and doubles. An examination of carefully stained slides leaves little room for question of the identity of these bacilli with some of those introduced with the food, but the interval was too short to make it certain that they had multiplied since ingestion. Their occurrence, however, in such vast numbers in the blood so soon after death, makes it very unlikely that they merely represented an escape of the intestinal fluids, especially as we shall soon see that the same bacilli occurred abundantly in the blood of larvæ not yet dead. The intestinal contents were full of the above Bacillus and the usual Micrococcus, 1 μ in diameter, in singles, doubles, and patches. The food contents were partially digested.

Besides the above bacteria, the blood was yellow with masses of cells with granular contents, many with a large nucleus each. These cells were apparently derived from the fatty bodies, which seemed to be in process of disorganization, but differed from the usual mulberry bodies which result from pupal histolysis, by the fact that there was no appearance of the division of the cell contents into mulberry granules.

Another larva observed this day, June 29, evidently torpid and apparently sick, seemed to have moulted imperfectly, fragments of the skin still clinging to the shrunken posterior segments. The body was flaccid, but not discolored. A proleg being snipped off, no flow of blood followed, but the fluid pressed out contained a moderate number of the above bacilli, no micrococci, but many well-defined mulberry cells and granules. Each of the cells contained from ten to fifteen or twenty of the latter. The alimentary contents contained micrococci with an occasional Bacillus, but none of the mulberry granules, both forms of bacteria being in this larva much

less abundant than was usually the case with individuals so seriously affected. The epithelial cells of the intestine contained granular masses, seemingly of the micrococci, and the fluid bathing them was thick with the same objects. Occasionally patches or clusters of the micrococci occurred in contact with the food. The stained and mounted slides of the blood show chiefly mulberry granules, spherical or somewhat angular in outline, 1.5 μ to 3 μ in diameter. A small number of spherical micrococci also occurred, many of them minute, ranging from .6 μ to .8 μ. These appear in all the usual forms of aggregation, including doubles, short chaplets, and patches of considerable size. Bacilli also occasionally occur, with parallel sides and rounded ends, from 1.25 μ to 1.5 μ in transverse diameter, and from 3 μ to 4 μ in length. A single *Bacillus intrapallens* was noticed in process of development, measuring 1.75 μ by 2 μ.

On the 30th June still another larva died, the grayish fluids of which contained immense numbers of the spherical micrococci, single and double, with vast quantities of the bacilli above described, — motionless at first, but soon, near the edges of the cover or in the vicinity of a bubble, commencing active flagellate movements. The body of the next larva to die, (July 1,) was flaccid, and contained little fluid. Immense numbers of spherical micrococci, 1 μ in diameter, occurred in the blood, mostly in doubles, together with many ovals about 1.5 μ long. Neither Bacterium nor Bacillus were detected in this specimen.

On the 2d, a caterpillar, evidently diseased, shrunken, and shortened, but with colors yet bright, was found lying upon the floor of the cage, able to right itself when turned over, but making no effort to escape. Blood from a foot of this larva contained a great number of unsegmented cells, similar to blood corspucles, but of variable size and shape, some with and some without nuclei. A few hours later, when the blood was examined again, besides these cells were found a considerable number of segmented bodies and mulberry cells, the latter evidently due to dissolution of the former. The next day this segmentation of the cells in question had gone still farther in this larva, and very many mulberry cells were distinguishable, together with others but partly segmented.

Now killing the larva, I found the fluids full of mulberry cells and granules, together, with a great number of spherical micrococci,—so determined by staining coagulated films.

On the evening of July 1 a number of larvæ in this cage were curiously affected, the prolegs, except the anal pair, being enlarged and swollen, with a slight reddish discoloration. These larvæ were evidently greatly annoyed by their condition, and dragged themselves clumsily about as if half paralyzed. One was seen to turn violently upon itself, and bite the swollen prolegs, as if in pain, so that the blood flowed from them freely. On the following morning one of these caterpillars was crawling about with the abdomen twisted and the prolegs turned almost upwards. Carefully snipping one of these swollen legs, I found in the blood an extraordinary number of lymphoid corpuscles, and a very considerable number of mulberry cells, but little, if any, larger than corpuscles of the blood, varying from circular to oval in optical section. Frequently a nucleus was visible in the midst of the mulberry granules, but no cell walls were distinguishable. The unstable character of the segmentation of these cells was unexpectedly demonstrated by the effect of a little carbolized water run under the cover. As a consequence, the segmentation entirely disappeared, the mulberry cells being all re-converted into simple nucleated corpuscles with granular contents. In fact, I happened to witness this retrogression of a mulberry cell, — a mass of distinct granules with a nucleus dimly seen among them, converted, with a curious internal commotion, into a common lymphoid corpuscle, of rather large size, with clearly distinguishable nucleus. In this condition the cells were indistinguishable from dead blood corpuscles. No bacteria were visible in these fluids.

On the 3d July one of these larvæ died. The body contained but little fluid, but this was loaded with cells, some unsegmented nucleated sphericals of various sizes, without trace of cell wall, staining deeply with aniline; and others well-developed mulberry cells, but so similar to the foregoing as to have been apparently derived from them. On the mounted slides of this material are also great numbers of separate mulberry granules and the usual spherical micrococci, the

latter averaging 1 μ in diameter, with an occasional Bacillus like those already several times mentioned. Micrococci and bacilli were, however, less abundant in these fluids than is commonly the case with larvæ destroyed by bacterial disease.

In a peculiar larva which died July 2, a small specimen that had scarcely grown since it was first placed in the cage, a few micrococci were found, and a considerable quantity of the mulberry granules, although this individual caterpillar must have been far from the pupal stage of development.

In another larva examined at the same time, likewise dwarfed, although larger than the preceding, the blood was gray with the usual Micrococcus, both free and in masses, and contained likewise great numbers of mulberry cells and granules. On the 12th July a larva died in whose blood no bacteria were detected, save a few of the usual bacilli. Its fluids contained, however, an immense number of mulberry cells and granules.

From the 12th to the 14th July eight more larvæ died in this lot with symptoms and microscopic characters like those already described,—the body usually somewhat shrunken and flaccid and the colors unchanged. The blood was occasionally gray with micrococci, but more commonly differed in appearance from that of healthy larvæ, only by the slightly yellowish or whitish tinge. The original Bacillus found in the earlier specimens occurred but once in these, and then in trifling quantity. The ordinary Micrococcus was more commonly present, sometimes, indeed, profusely abundant, but at other times in relatively trivial numbers. The unvarying and characteristic feature was the number of free cells in the blood, of variable form and size, some of them being altered blood corpuscles and others evidently derived from the fatty bodies. These occurred in all stages of segmentation, from a mere trace of commencing subdivision to a complete separation of the entire contents of the cell into more or less equal granules. The absence of an enclosing wall was unquestionably evident, granular masses being occasionally found from which a single one of the mulberry granules had broken away, leaving the remainder undisturbed. When the segmentation of these cells was incomplete or indefinite, they readily reverted to nucleated cells with gran-

ular protoplasm, if treated with alcohol or carbolized water. In many of the mulberry cells the nucleus persisted, surrounded and obscured by completely formed granules, but in others this seemed likewise to have participated in the metamorphosis of the body of the cell. The number of granules in a single cluster varied from three or five to fifteen or twenty in an optical section of the mass. The few remaining larvæ of this lot were now transferred to alcohol and glycerine for histological study.

In the meantime matters had taken a somewhat unfortunate course in the so-called check lot, these larvæ commencing to die mysteriously on the 30th of June. The first victims were two dwarfed specimens which had evidently moulted very imperfectly, being still covered with fragments of the old integument. An examination of the fluids of these specimens afforded no explanation of their death, as they contained neither bacteria in any appreciable number nor any cellular bodies. Another affected larvæ proved to have been parasitized.

Next two larvæ were found dead upon the morning of July 3, the fluids of which were grayish in hue. These contained no recognizable bacteria whatever, but were loaded with segmented mulberry cells.

On the 10th of July a larva died whose blood contained a moderate number of micrococci in doubles and chains, concerning which no further notes were made at the time and the slides illustrating which were lost.

A larva evidently diseased on the evening of this day was noticed the next morning with several spherical masses of excrement clinging to the vent, connected with each other by a delicate film. This film was dissected off, stained and mounted, and found to consist of an exceedingly delicate, structureless, but rather firm, membrane (doubtless the cuticle of the intestine) through which were dispersed great numbers of micrococci,—unquestionably a pure culture. These were mostly collected in patches, some compact and well defined, others more or less diffused. The compact clusters varied in outline from nearly circular to elongate oval. One of the latter was 35 μ long by 8 μ wide; others were respectively 18 μ by 20 μ, 16 μ by 16 μ, and 12 μ by 20 μ. The micrococci composing

them were 1 μ in average diameter, slightly oval to the eye, though not measurably so.

On the morning of the 12th two other larvæ were dead. The blood of one contained only immense numbers of mulberry granules with a moderate number of possible spherical micrococci, — not positively distinguishable in our slides, however, from the smallest mulberry granules. The blood of the other larva was in a similar condition, heavily loaded with mulberry cells and the results of their disintegration, but contained, likewise, a small number of various bacteria,—rarely a short, broad Bacillus, apparently identical with that first used in the experiment ; more abundantly a small spherical Micrococcus, differing in appearance from the usual form ; also a double oval Micrococcus, and an occasional patch of the true spherical so abundant in these experiments. These last were sometimes associated on the slides with patches of unsegmented cells, which evidently had their origin in the fatty bodies.

The third larva dead this day was soft, shrunken, and nearly dry. The scanty fluids were full of micrococci and thick with mulberry cells and granules. The effect of carbolized water upon the cells was, in this case, to cause separation into their constituent particles.

The results of all the above observations and experiments upon the zebra caterpillar may be summarized as follows : At least one of the bacillar forms occurring in the culture used in this infection was conveyed to the larvæ under experiment with fatal effect, and probably multiplied there successfully. This Bacillus almost wholly disaapeared, however, in the later stages of the experiment, and so is not certainly a true pathogenic form. Associated with this in the fluids of the larvæ treated were the usual spherical micrococci of this disease, clearly identical with those applied to the food, and certainly multiplying freely in the bodies of the larvæ. These presented, consequently, the characteristics of a pathogenic microbe. A curious change was observed in the phenomena of the disease in the experimental lot. Death seemed at first occasioned by the immediate action of the bacteria ingested or cultivated in the blood and alimentary fluids ; but at a later period after the

infection, these bacterial forms became less abundant, and the blood was loaded with the products of histolysis, partly, in all probability, of the blood cells and partly of the fatty bodies. There seems to have been in general an inverse relation between the abundance of the bacteria and the abundance of these histolysis products, the former becoming less numerous with lapse of time and the latter more so. These facts have an interesting application to those observed in the silkworm, as detailed on previous pages, the condition of the later examples of the zebra caterpillar being, in fact, almost precisely similar, so far as microscopic appearances go, to that of silkworms supposed to be suffering from jaundice.

I have, consequently, to suggest a similar explanation of these phenomena; viz., that in the case of the latter larva the bacterial affection largely lost its power, but still retained sufficient energy to overthrow the physiological balance as the larvæ approached the age of pupation, death resulting from the premature histolysis of certain of the larval structures, — notably the fatty bodies.

The history of the check lot gives no evidence of serious bacterial infection, but rather of that modified form of it which produces premature pupal histolysis. Reviewing the entire series of slides and cultures, I have no doubt that these indicate the successful preservation through the winter and transference to the bodies of the zebra caterpillars of certain of the forms characteristic of *flacherie* in the walnut caterpillar, *Datana angusi.*

THE EUROPEAN CABBAGE WORM.

(Pieris rapæ, L.*)*

A second infection experiment was begun with the same fluids as the foregoing upon fifty cabbage worms, twenty-five of which were selected for treatment, and an equal number isolated as a check.

On the 6th August, four days after the infection, a larva was found dead upon the bottom of the cage. On puncturing the back a clear, greenish fluid exuded, which was swarming with a large and very active Bacillus, occurring usually in

doubles. Stained slides of this exhibit the same characteristics as those made directly from the culture used for the infection, but nothing else is evident.

On the same day another larva was found dead and blackened, clinging to the side of the cage, in quite different condition, however, from cabbage worms affected by their own peculiar disease. The body contained but little fluid, and that was of a paste-like consistence, full of the above bacilli, which the mounted slides show to be an absolutely pure culture.

Another larva, which died the following day, August 7, was found to present precisely the same microscopic characters, only large bacilli occurring in the slide. By the 10th ten of the specimens under experiment had either pupated or were evidently making preparations for that change. But two were apparently diseased. One of these last perished on the 12th, its body soft, pale, blackened posteriorly, but not deliquescent. The blood contained a multitude of minute spherical granules, some Bacillus-like structures, more slender than those previously occurring, and also floating cells of the fatty bodies containing mulberry granules, irregular in size, and sometimes showing also a central nucleus. With these were many large micrococci, 1 μ in diameter, circular, or sometimes slightly oval, commonly in singles or doubles, with rarely a chaplet of four. This larva soon became deliquescent, as if affected by the original *flacherie* of the cabbage-worm ; its condition, in fact, indicating a mingling of two diseases,—that conveyed by the infection to the larvæ, just described, and the one native to the species. It will be noted that one of the effects of the original infection seemed already to have waned, and that the development of the mulberry cells and granules characteristic of this condition had already occurred, — a phenomenon especially significant, since in the native disease of these cabbage caterpillars no similar condition of the fluids was ever seen. Another larva, dead this day, presented appearances so precisely similar to the preceding that no special description of it was made. The check lot, in the meantime, had progressed without injury. August 14 this experiment was interrupted, owing to a discovery of the fact that, through some oversight of the attendant, the full number of the larvæ placed

in the breeding cages could not be accounted for, several having, apparently, been allowed to escape as the food was changed. This partial experiment can, consequently, only be held to verify the conclusion drawn from the one just previously described, to the effect that the Bacillus used for infection may be at least temporarily propagated in healthy larvæ with destructive effect. It is proper to add that in the remnants of both the infected and check lots, the common *flacherie* of the cabbage worm afterwards broke out, showing that these insects had been exposed to this disease before they were brought to the office for the experiment.

Muscardine.

This disease, long well known in the silkworm, is not a bacterial affection, but is due to an invasion of the body of the insect by the filaments of a "thread fungus" (Hyphomycetes), whose spores germinate on the surface. These send thread-like processes through the skin which at first bud off from their free ends, within the body, short cells (sometimes called "conidia") with which the blood of the diseased insect speedily becomes loaded. These multiply by division, and finally result in a thread-mycelium which makes its appearance on the surface of the insect, and bears vast numbers of spores, white or green, with which the body becomes covered as with a fine dust. An affected larva is commonly flaccid and shrunken at death, but finally, as a consequence of the *post mortem* development of the fungus, becomes filled with threads and spores, and distended to its original size, drying without shrinkage into a hard and brittle mummy.

These later stages of the development of the fungus are greatly affected by the weather, a drouth preventing the conspicuous external appearance of the mycelium and the development of spores, and thus limiting the spread of the disease.

Every experienced collector finds occasional examples of this disease in the field in the form of stiff and mummified insects, often covered with a dense white or greenish bloom; but few observations of any wholesale destruction of a super-abundant species by it have been recorded, — none for America

as far as I am aware. The following observations on the history of a tremendous outburst, in southern Illinois, of a species of caterpillar, one of the most destructive insects known, and of the means by which this irruption was apparently terminated, will consequently be of considerable interest.

In April and May, 1883, the extreme southern part of the state, from Cobden southward, was the scene of one of the periodical uprisings of the forest tent caterpillar (*Clisiocampa sylvatica*), which have doubtless occurred at intervals in that region from time immemorial. Vast numbers of forest trees in the southern counties of Illinois and in the adjacent parts of Missouri and Indiana were as completely defoliated as if mid-winter had suddenly burst upon them in May, and whole orchards of many acres of apple trees were left without a single green leaf. Oak, hickory, the black and sweet gum, and dog-wood were the trees especially selected for destruction in the forest, and the apple on the fruit farms, — the foliage of the peach being scarcely touched, even when the trees were covered with the caterpillars. Strawberry fields were likewise vigorously attacked, — young fields being occasionally nearly eaten up.

By the 18th May, when my visit there was made, the larvæ had nearly all attained their growth and were travelling restlessly about by myriads, in every direction, in search of suitable places for pupation,—a few having, in fact, already transformed along the tops of fences and under rubbish on the ground. As I walked along the road sides my attention was immediately caught by the great numbers of dead larvæ dried against the boards of the fences, usually in a vertical position, and the multitudes apparently in a diseased condition, traveling more or less feebly, or resting motionless with the head downward. These larvæ were usually flaccid and shrunken posteriorly, but not especially discolored.

It was, unfortunately, impossible for me to make any careful examination of the disease at this time, and no other opportunity offered during the season.

Revisiting this region on the 11th July, an assistant found that the moths had all emerged sometime previously, but that from one half to three fourths of the cocoons had never yielded

the imago. From a few of these, parasites had evidently escaped, but in most cases there was nothing in the external appearance of the cocoon to explain the failure of its development. Returning to this region June 3d of the following year, we learned from A. J. Ayers, Esq., of Villa Ridge, that a sufficient number of larvæ hatched that spring to do considerable damage, but that when they were a little over one half an inch long they died and dried upon the leaves, sometimes whole colonies being found dead together. Occasional examples of larvæ in this condition could even then be found on the apple-trees. A few apparently healthy examples were collected at this date and brought to the Laboratory at Normal. These were carefully fed and attended, with the expectation of obtaining the imago, but all died, without exception, with symptoms precisely resembling those of the year before, as they then came under my observation.

The first of these larvæ was seen to be sick on the 27th June, ejecting from the mouth and vent a fluid which contained great numbers of oval corpuscles, not unlike those characterizing *pébrine*, but varying appreciably in size and shape. Examples were found in process of sub division, or even, in occasional instances, short strings of three not wholly separated; and other examples occurred where a spherical lobe was borne upon the end of an oval cell, as if the latter were budding endwise. All these appearances were inconsistent with the hypothesis of the presence of *pébrine*, the characteristic "corpuscles" of which develop by internal segmentation of spherical masses (Sporozoa) and are never connected in doubles nor multiply by fission. Dissections of these larvæ afforded evidence that they were attacked by muscardine. In specimens which had lain some time it was not difficult to identify a scanty mycelium in the body, although, owing probably to the dry and warm weather at this season, there was no external development of the fungus either in the form of threads or spores. These larvæ continued to die until July 5, at which time the last perished.

The individual cells found in the blood varied from 2 μ to 3.5 μ, and in length from 3.5 μ to 5 μ. They differed also in shape, some being a rather broad symmetrical oval, and others

narrower towards one extremity. Nuclei about one half as long and wide as the cells containing them were visible in most. Neither cells nor nuclei stained readily with aniline.

The blood of many of the larvæ examined contained also considerable numbers of mulberry cells of rather large size, composed of granules averaging about 2 μ in diameter.

As no insects affected by muscardine had been handled by us at the time these caterpillars were received at the office, it is certain that they brought the infection with them; and as all perished, without exception, from this same disease, and this without the development of spores by which the contagion might have been conveyed from one to another, the presumption is very strong that the affection illustrated by these individuals was that which had swept away the greater part of the entire brood of the preceding year, and especially that which had caused the death of the young larvæ as reported by Mr. Ayres.

SUMMARY AND CONCLUSION.

The circumstances under which the studies above described have been made; the fact that they belong to a field of research so difficult that new .comers are very properly viewed with a certain suspicion until they have clearly demonstrated their right to labor in it; and the further fact that my results have not always emerged from the cloud of experiment with perfectly clear and definite outline, have seemed to me to require in this paper a quantity of detail sometimes amounting, perhaps, to wearisome prolixity: and the following summary of the principal features and results of my research has been prepared in the hope that it may serve to make this mode of treatment less objectionable.

I have first attempted to characterize a common and highly destructive disease of the European cabbage worm (*Pieris rapæ*), by whose ravages the injuries of these pests have received a very important check,—a disease especially marked by the whitish color of the living larvæ, amounting before death to an ashy or almost milky hue, and by a rapid *post mortem* blackening and decay. The distinguishing microscopic appearances are, first, a remarkable whiteness and opacity of the circulating fluids which are early loaded with immense numbers of very

minute spherical granules from .5 μ to .7 μ in diameter, staining with aniline fluids, although sometimes with difficulty, and less highly refractile than ordinary micrococci; second, a great degeneration of the mucous membrane of the chiliferous stomach producing before death a marked diminution in the thickness of the epithilial layer; and third, the appearance in the alimentary fluids, and usually also in the blood, of sphericals and ovals (especially the former), presenting every characteristic of unmistakable micrococci. Few if any of the blood granules are affected by ether, and they dissolve in hot caustic potash little, if at all, more readily than known micrococci, bacilli, and bacillar spores,* but they are not all of them certainly to be understood as of bacterial character. The fatty bodies are the next organs to suffer, after the alimentary canal, and speedily undergo an immense degeneration.

That this disease is contagious is shown by its unequal distribution in the neighborhoods affected by it; by its gradual though rapid progression from one part of the field to another; by its evident independence of locality, climate, and weather; by its apparent progress across the country from east to west; by the probable success of experiments made to convey it from infected regions to others at a distance, not previously invaded by it; and, finally, by its evident bacterial character.

In 1883 and 1884, numerous cultures were attempted in beef broth by the strictest methods of fluid culture in tubes and flasks, the accuracy of which was attested by the fact that the check tubes in every instance remained unchanged throughout. Not all the cultures were successful,—several careful infections from the blood especially being without result; in other cases, however, such infections from the blood of still living larvæ yielded the spherical micrococcus figured in the plate, identical in appearance with that observed in the fluids of the diseased larvæ, but larger in average size than the supposed

*Contrary to the statement frequently made respecting the effects of alkalies upon bacteria, I have found that hot solutions of caustic potash rapidly attack both the cells and spores of *Bacillus subtilis* and the common micrococci of fermentation. Two or three times heating to a boiling point in a strong solution is sufficient in most cases to completely destroy these microbes.

blood form. Cultures from the alimentary fluids were never without result, although occasionally impure; but the commonest forms there were micrococci like the above, and the next commonest an oval micrococcus of nearly the same size and general appearance. Specimens of Bacillus and Bacterium were frequent in these alimentary cultures, but far less constant than the micrococci. No opportunity offered for experimental infection of healthy larvæ of this or other species with the cabbage worm microbes, either native or cultivated, and consequently it must be confessed that, strictly speaking, the proof is incomplete that this affection of the cabbage worm is a germ disease, although it certainly amounts to very strong probable evidence.

More complete and conclusive studies were made of a disease of the silkworm apparently identical with that known to the French as *jaunes*, and called jaundice by English and American writers. This disease, distinguished especially to the eye by the decided yellow color and restless activity of the larvæ, by the tender skin, easily broken, and by the free flow of thin yellow blood, is microscopically characterized by an abundance, in the blood, of the spherical or polygonal granules and clusters of the same, resulting from the peculiar degeneration of the larval tissues proper to pupation, — these being in this case derived chiefly from the fatty bodies and in part also from the blood corpuscles. This disease, therefore, seems to be essentially a premature pupal histolysis of the fatty bodies,—or, more properly, to be due to a retardation of the pupation of the larva which takes unequal effect on the different tissues, the fatty bodies breaking down before the muscles and membranes are ready for pupal transformation.

Spherical micrococci .75 μ to 1 μ in diameter occur in the walls of the alimentary canal as accompaniments of this disease, and are believed to be one, at least, of the exciting causes of it, although it seems not impossible that other retarding influences may produce a similar effect in overthrowing the normal physiological balance as pupation approaches.

That this supposed jaundice was contagious, was shown by the phenomena of its occurrence at Champaign, and that the bacteria accompanying it were capable of exciting disease in other larvæ was proven by first cultivating them repeatedly

in beef broth and then producing in cabbage worms *(Pieris rapæ)* a similar disease by moistening their food with the culture fluids containing the bacteria. While this disease, artificially induced, in some cases came so near that of the native cabbage worm as to suggest that the bacterial treatment served only to excite the natural disease of the larvæ, in other cases it was clearly different from the above and presented characters so clearly like those of the silk worm *jaundice* that there could be little doubt of an actual transference of the original disease, especially when the blood of the sick cabbage worms was found loaded with the mulberry cells and granules of pupal histolysis.

I have next reported at length on a breeding-cage disease attacking the YELLOW-NECKED APPLE CATERPILLAR *(Datana ministra)* and the WALNUT CATERPILLAR (*Datana angusi*), so similar to the well-know *flacherie* of the silkworm that I have not hesitated to call it by that name. Its principal symptoms are those indicating a gradual weakening of the larvæ, usually accompanied by brownish fluid discharges from the vent and a consequent shrinking and softening of the body. The alimentary canal contains always great numbers of microbes, commonly of considerable variety,—including bacilli, bacteria, and micrococci, the most abundant and characteristic being oval and spherical micrococci not distinguishable from those mentioned above. The method of the appearance and spread of the disease in our breeding room indicated a contagious character; and this conclusion was verified by culture of some of the bacterial forms encountered and their successful use as an experimental virus.

The cultures (in beef broth and on thin gelatine films) related to both micrococci and bacilli, and both were preserved over winter in plugged test tubes and in small sealed tubes, cultivated the following season, and applied to the food of another species of larva,—the ZEBRA CATERPILLAR (*Mamestra picta*). The first result of this treatment was the destruction of several of the larvæ, in from two to six days, with a disease marked by the appearance in their intestines of great numbers of bacilli (in the specimens first to succumb) and micrococci (later). The affection seemed then to change its character to one resembling jaundice of the silkworm, the characteristic

histolysis granules commencing to appear in the blood of slightly affected larvæ as early as the fourth day after infection. Caterpillars thus attacked did not commence to die until the sixth day, and most lived until the 15th. As in the case of the silkworm jaundice with which this is compared, the bacterial affection was less evident than in more rapid and pronounced cases of disease, but the usual intestinal micrococci were always present in varying numbers.

The last infection experiment I had to report, began August 2, 1884, with the same fluid, applied to the food of the European cabbage worm, was abandoned August 14 because the assistant in charge was unable to account for all the larvæ,—some having evidently been allowed to escape when the food was changed. As far as carried, it tended to confirm the indications of the preceding experiment, the blood of those dying up to the 7th August being full of a large active Bacillus only, similar to that used in the infection, and those perishing later containing chiefly large micrococci together with mulberry cells and granules. Later the common *flacherie* of the cabbage worm appeared in the remnants of both the infected and check lots.

Finally in a note on muscardine I have attributed largely to this affection the disappearance of a vast host of the forest tent caterpillar (*Clisiocampa sylvatica*) which devastated the forests and orchards of a part of southern Illinois in 1883, basing this conclusion upon the observed phenomena of the disease appearing among them as compared with those accompanying the death of larvæ of this species from the same localities, perishing in our breeding cages the following year of demonstrated muscardine.

There now remains to me only the pleasing duty of acknowledging my grateful obligations for aid in this work to my first assistant, Mr. W. H. Garman, to whose faithful care and unimpeachable accuracy of manipulation the larger part of the bacterial cultures were due; to Prof. T. J. Burrill, who has had the kindness to examine many of my slides, giving me the benefit of his extensive acquaintance with the bacteria; and to Dr. H. J. Detmers, now of the State University of Ohio, to whom I owe, among many other favors of this character, the excellent photographs of micrococci reproduced in the plate.