covered; only an examination of the work itself can show the method of treatment and the completeness with which the many phases of the many-sided microbiology are treated.

The book is well printed, though the type is small and the pages look crowded. There are 128 figures in the book, of widely varying grades of merit. The editing is well done and the errors are few. Whether or not the book will prove useful in classes it will be indispensable for a bacteriologist's book shelves.

H. W. CONN

SPECIAL ARTICLES

STUDIES ON THE WILT DISEASE, OR "FLACHERIA" OF THE GYPSY MOTH

FOR the past six months we have been engaged in a study of the cause and nature of the wilt disease of gypsy moth caterpillars. The disease, so far as we are able to learn, is similar to the one attacking the nun moth (*Lymantria monacha* L.) in Germany. But although the investigations carried on in that country have led usually to negative results so far as the causative agent of the disease is concerned, still the work has been in the main of a scientific character. We are speaking of such work as has been done by Escherich, Prowazek and Tubeuf. This is more than can be said of some of the attempts made in this country and we thoroughly agree with Escherich,[1] who says, in speaking of a recent paper by Mr. William Reiff[2] "Es fehlt also so ziemlich alles, was zu einem wissenschaftlichen Beweis für die behaupteten Zusammenhänge gehört."

Our first attempts were confined to a search for protozoa in the tissues of the caterpillars, and while dissecting and examining these many were seen to contain certain polygonal bodies clustered around their tracheæ. These bodies have a very high refractive index and resist all stains, with the exception of iodine, in which they take on a uniform tint. No definite internal structure can be detected, however, and it finally dawned upon us that we had a case here analogous to the one in the nun moth. Bolle[3] first found these bodies in sick silkworms, and Tubeuf later discovered them in nun moth caterpillars afflicted with the "Wipfelkrankheit," a sickness the symptoms of which seem to be in many respects similar to those of the gypsy moth wilt. Wachtl and Kornauth[4] were the first to realize that the so-called polyhedral bodies have a diagnostic value, for caterpillars afflicted with "Wipfelkrankheit" are never free from them. Wolff[5] thinks that they are reaction-bodies having nothing to do with the cause of the disease. This he believes to be due to the presence of certain bodies called "Chlamydozoa" by Prowazek. Wolbach and McKee,[6] however, have since shown that the "Chlamydozoa" are products of mucous secretions under pathological conditions and not organisms. Escherich and Miyajima[7] resumed the study of the polyhedral bodies and besides presenting many original observations, confirmed Wachtl and Kornauth's results as to the high diagnostic value of these crystal-like aggregates. The figures and descriptions given by the former authors are very good, and we have no reason to doubt that the bodies which we find in the gypsy moth are precisely the same. At the beginning of the infection these polyhedral bodies are few in

[1] *Naturwiss. Zeitschr. für Forst und Landwirtschaft*, Heft 2 u. 3, Feb.–Marz, 1912, p. 85.

[2] "The Wilt Disease, or Flacherie of the Gypsy Moth," published by the Bussey Institution of Harvard University, 1911.

[3] "Der Seidenbau in Japan, nebst einem Anhang: Die Gelb-oder Fettsucht der Seidenraupe, eine parasitäre Krankheit," Budapest, Wien und Leipzig (Hartlebens Verlag), 1898.

[4] "Beiträge zur Kenntnis der Morphologie, Biologie und Pathologie der Nonne," *Mitteil. forstl. Versuchswesen Österreichs*, Heft XVI., Wien, 1893.

[5] "Uber eine neue Krankheit der Raupe von *Bupalus piniarius* L.," *Kaiser Wilhelm-Institutes für Landwirtschaft in Bromberg*, Band III., Heft 2, 1910, s. 69–92.

[6] "The Nature of Trachoma Bodies," *Journ. Med. Research*, n. s., Vol. XIX., No. 2, pp. 259–264, April, 1911.

[7] "Studien über die Wipfelkrankheit der Nonne," *Naturwiss. Zeitschr. für Forst und Landwirtschaft*, Heft 9, 1911, pp. 381–402.

numbers, but later they surround the tracheæ in curious cyst-like clusters. Still later the other cells become filled up with them and finally, when the caterpillar dies and disintegrates, they escape into the body fluids. The polyhedral bodies, to be sure, behave as crystals, but, not finding at the time anything of interest in the tissues, we gave them considerable attention, confirming Escherich's various chemical tests and staining reactions. That the polyhedral bodies might be organisms, perhaps distantly related to the microsporidians, seemed inconceivable; still their curious cyst-like arrangement around the tracheæ helped much towards concentrating our studies upon them. They revealed nothing, however, which could in any way be associated with parasitism and were finally abandoned as mere reaction-bodies, possibly urates. They react fairly well to the murexid test, giving all the color reactions except the last one. Why we have been unable to obtain this last reaction we are at present unable to say. We find that these bodies can be readily centrifuged out from sick and dead caterpillars and in quantities sufficient for purposes of chemical analysis, and we hope to be able to give a more intelligible account of them later. Nevertheless, as has always been supposed, the polyhedral bodies seem to have some significance, for after using good light and very high magnification small wriggling organisms were observed in the fat cells and other cells at such times as the polyhedral bodies were clustered around the tracheæ. These moving organisms were stained and found to be bacteria. From this time on we pursued the work along bacteriological lines and we believe have been able to demonstrate the etiological connection of these bacteria with the disease.

Living caterpillars are the only ones which can be treated with fixing fluids for sectioning. When a caterpillar dies of the wilt the degeneration of the tissues is so rapid that it is impossible to handle it. When touched, it goes all to pieces and therefore can never be used for histological work. Some of the sectioned material showed that hardly any of the tissues failed to reveal the presence of this bacterium. It was found in great numbers in all parts of the intestine and in many caterpillars appeared to be in the act of perforating its walls. The fat cells seem to be particularly liable to attack, which probably accounts for the saponified nature of the fat of sick caterpillars. The musculature, ganglia, testes, ovaries, œnocytes and other cells are also heavily parasitized. In fact, as previously stated, nothing seems to be exempt, since the infection extends even to the hypodermal cells. Some larvæ show a heavier degree of parasitism than others, while a certain number apparently not diseased may be free from the bacteria.

The organism in question is very small, having a diameter of only .51 μ–.85 μ. It resembles *Pneumococcus* very closely except that it is motile, progressing in a gyrating manner. For this reason, and because it seems to be an undescribed form, we have named it *Gyrococcus*. A brief technical description of it is given at the end of this paper.

Smears of dead larvæ were now studied and after making the smears very thin and using Grübler's Giemsa or Delafield's hæmatoxylin with eosin, the *Gyrococci* were more clearly revealed. Owing to their minuteness it is almost impossible to see them in thick smears. They are also apt to be obscured by the polyhedral bodies, which are very abundant in dead material, so that smears must be thinned out with sterile water in order to separate these bodies. Then only can the *Gyrococcus* be recognized easily under the 2 mm. oil immersion in combination with the compensating 12 or 18 ocular. In material which has been dead a long time many different species of septic bacteria accumulate, but caterpillars which have just died are fairly pure except for the *Gyrococci* which are present in large numbers.

In order that the non-pathogenic forms might give us as little trouble as possible, we inoculated sterile veal tubes with the fat of living infected material. These tubes were kept in an insectary where the temperature fluctuated during May, June and July between 80° and 95° Fahrenheit. We thought that

such a temperature might be favorable for growth, because we found that a certain number of our caterpillars died from "flacherie" throughout the winter whenever we allowed the temperature to rise sufficiently in the insectary. Furthermore, our field experience later in the season clearly demonstrated to us that more caterpillars die of "flacherie" on a warm than on a cool day. In twenty-four hours the veal tubes became turbid. They were examined and *Gyrococcus* was found to be present in great numbers, together with a few other forms. These were then isolated on agar and after about forty-eight hours, small, round or oblong, smooth, cretaceous colonies were found, which showed again after microscopic examination that our bacterium grows on agar. Other sterile tubes were inoculated with these pure colonies and after twenty-four hours more a pure growth was obtained. These tubes remained odorless from the beginning of the growth to the time when the nutriment became exhausted. The first impure set of tubes had an odor due to the presence of septic forms.

For inoculation purposes, the fact that larvæ looked healthy externally was not considered to be sufficient evidence that they were free from infection, for a larva may appear reasonably healthy and feed normally with a greater or less number of germs in its system. As a matter of fact, if the temperature and food conditions are favorable a larva may pass through its sixth moult, pupate and even transform into a moth, carrying a number of *Gyrococci* along during the process. External conditions have a great influence on the rapidity with which the *Gyrococcus* multiplies within its host and for that matter within the veal tubes also. Hence, owing to the apparent feeble virulence which the *Gyrococcus* has when few in numbers, the external appearance of a larva means absolutely nothing. The blood, however, affords a very excellent diagnostic medium. The caterpillars were therefore tapped and only those were used in the experiments which were found to be free from the *Gyrococcus*. The blood was usually tapped from one of the prolegs. This operation can be repeated on the same larva at intervals of a day or two without injuring it. After tapping the blood each caterpillar was isolated in a separate, clean box and fed only with food which had been carefully selected and washed.

All the caterpillars which were pronounced free from infection after the blood had been carefully examined for *Gyrococcus* were divided into four lots. Each lot was used for an experiment with a pure culture of the bacterium. Twelve caterpillars were inoculated in the proleg and twelve in the dorsal vessel. Four controls accompanied each one of these lots. Twelve caterpillars were fed with the pure culture from a sterile pipette and fourteen were fed with leaves smeared with the culture.

The table given below comprises the results of one series of our experiments. The rate of death at each day succeeding the inoculation or the feeding is represented. Since at this time all of the caterpillars were full grown several of them pupated.

TABLE

The first and second days were very hot.
The third and fourth days were very cool.

Number of Days After Inoculation or Feeding	No. of Caterpillars which Died			
	Inoculation in Proleg	Inoculation in Dorsal Vessel	Fed from Pipette	Fed with Smeared Leaves
First day	3	3	3	6
Second day	2	2	4	2
Third day				
Fourth day				
Fifth day	1	1	3	2
Sixth day	2	1	1	
Seventh day		1	1	2
Eighth day				1
Ninth day		1		1
Tenth day	1			
Eleventh day	1	1		

It will be seen from the table that ten caterpillars out of the twelve inoculated in the proleg succumbed to the disease. Two out of the ten died in the pupal stage. Of the surviving two, one is still a pupa at the time of writing and one emerged. Ten out of the

twelve inoculated in the dorsal vessel died, the remaining two are still pupæ. All of the deaths were typical of "flacherie." Of the two lots used for feeding experiments all died without a single exception. All of the controls survived and pupated, and some of these have already become moths.

It will be noticed that some caterpillars seemed to be more resistant than others or perhaps they may not have received as large a dose and consequently were longer in dying. The feeding experiments were particularly successful, all of the caterpillars succumbing within nine days with the typical symptoms of "flacherie." We performed several series of experiments similar to the ones outlined above and the results in general agreed very well with those of the described series. That the feeding experiments were more successful than the others may be accounted for in one of two ways. First, in feeding we evidently gave them a larger dose, for our inoculating needles are very fine indeed to avoid injury to the caterpillars and consequently the number of *Gyrococci* introduced by inoculation must be considerably less than the number introduced by feeding. Second, the infection naturally enters by way of the mouth with the food, for sectioned material shows that in some cases while none of the cells are as yet attacked, the proctenteron is nevertheless heavily infected. As soon as a caterpillar died it was carefully examined and those which died as a result of the two methods of feeding were found to be much more heavily infected than those which died after the inoculating experiments, although in the latter case the number of bacteria was great enough to have caused death.

Whenever any of the control experiments died, which did not often happen, we could always trace it to carelessness on our part, for careful reexamination of our original blood slide sufficed to show that the *Gyrococcus* had been present and had been overlooked. In nearly all cases we were successful in obtaining moths from our controls.

It was very interesting to see the influence which the temperature exerted on these experiments, for on hot days three times as many caterpillars died of the disease as on cool days. Two such cool days are represented in the table by the third and fourth dates, when not a single caterpillar died in any of the experiments. We do not mean to say that temperature is the only factor which is of importance, but its activating power is as striking in the laboratory and insectary as it is in the field. For this reason we believe caterpillars in their first, second and third instars usually escape not because they are small, but because when the caterpillars are still in these stages the weather is comparatively cool and the food is still plentiful even in heavily infested localities. Bad food or lack of food also bears an intimate relation to the period of life at which a caterpillar may die of the disease. Poor or insufficient food must obviously lower a caterpillar's vitality and weaken its powers of resistance.

In each series of inoculating experiments a few caterpillars survived and pupated. Some died with the disease during this stage, while a few transformed. When the latter were examined the body fluids and tissues of the moths were found to be full of *Gyrococci* and the ovaries were also infected, showing the great possibility for the transmission of the disease to the offspring through the eggs. In fact, there are two things which suggest that "flacherie" may be transmitted in this manner. First, caterpillars which are reared from eggs and kept isolated contract the disease independently of one another. Second, we have found the ovaries of material used for experimentation as well as some of the ovaries dissected from moths caught in the field to be infected. In the males, however, we have been unable thus far to find *Gyrococcus* either in the seminal fluids or in the spermatozoa. Hence we conclude that the transmission of the disease is probably accomplished through the eggs, although up to this time we have not had time to section any of these.

To exclude the possibility of having really inoculated an ultra visible virus together with the *Gyrococcus,* a large number of caterpillars

were prepared (blood tested) for inoculation and feeding with material passed through the Berkefeld filter. Those fed and inoculated with the filtered culture all survived, while those which were treated in the same manner with the unfiltered culture all died.

It might be well to call attention to the fact that caterpillars fed with the juices of those which die of the disease succumb as rapidly as do caterpillars fed with the pure culture. The disease is probably spread in nature by the juices of disintegrated caterpillars flowing over the leaves which are later eaten by others. We have found *Gyrococcus* in the fæces, and the fact that such excretions are washed over the leaves by rain seems to show that the disease may also be spread in this manner.

What economic value the "flacherie" disease may have in combating the gypsy moth, we are not prepared at present to say. We have no experimental evidence whatsoever that the disease may be air-borne, as claimed by Mr. Reiff, although, of course, we do not wish to exclude such a possibility. Our experiments seem to show, first, that it takes a good many *Gyrococci* to kill a caterpillar and, second, that conditions must be favorable for the disease or, putting it in another way, unfavorable to the caterpillars by lowering their vitality; so it seems very improbable that any such methods as are at present utilized for the artificial spread of "flacherie" will be of any avail.

The present race of gypsy moths in Massachusetts seems to be permeated with the disease, for we have been unable to find a single locality where "flacherie" is not accomplishing some good. Professor Wheeler has made many observations and he agrees with us as to the above statement. He has been out with us on many of our inspection trips and has likewise noticed the great influence which temperature and other external conditions seem to have on the disease. Since we can not control external conditions and since the disease accomplishes so much good in nature and is probably increasing year by year, and since it is transmitted from mother to offspring, we may have to content ourselves with its natural havoc.

In conclusion we append a description of the peculiar bacterium which we believe to be the specific cause of the wilt disease, with a discussion of its generic characters.

Gyrococcus flaccidifex gen. et sp. nov. Cells in free state spherical, becoming slightly oblong just before division. Division in one direction of space only. After division each half may be spherical or may come to an abrupt tip, assuming a more or less heart-shaped appearance. Frequently the two halves are unequal; one half may be spherical while the other may be more or less heart-shaped, or slightly oblong. If cells remain connected after fission, chains of three or four are formed. A chain exceeding four units has never been observed. Size of single cells; diameter .51 μ–.85 μ. No evidences of endospore formation. Capsule distinct. Organs of locomotion present. Gram-negative. Colonies on agar spherical or oblong, small (diam. .5–1 mm.), smooth, cretaceous.

All of the above size and form characters may vary somewhat, especially in *Gyrococci* under cultivation. They are somewhat larger after long culture on artificial media and the formation of chains of three or four is much more frequent. Indeed, the formation of chains has never been observed in the fluids or tissues of the host. We are certain that the *Gyrococci* are motile, for when all air currents are excluded from the slide with sterile vasaline and everything is quieted down as much as possible, they gyrate across the field of vision in all directions and with remarkable rapidity. Furthermore, their behavior in the living cells is such that no one would mistake their activities for Brownian movement. Flagellar stains were tried, but so far without success.

It will be seen from the above description that *Gyrococcus* resembles *Pneumococcus* more closely than any other form. Its motility and its negative reaction to Gram's stain are, however, sufficient, we believe, to exclude it from that genus and hence, owing to its peculiar gyrating mode of locomotion we have

proposed for it the generic name of *Gyrococcus,* and owing to the striking flaccidity of caterpillars dying as a result of its presence, we have selected the specific name *flaccidifex.*

A much more detailed account of our work will be published later.

R. W. Glaser,
J. W. Chapman

Bussey Institution,
Forest Hills, Mass.,
July 22, 1912

THE PROLIFICNESS OF GAMBUSIA

On June 3, 1912, there was received at the aquarium of the Bureau of Fisheries in Washington a lot of top minnows (*Gambusia affinis*) from the lower Potomac River, comprising several males and about 90 females heavy with young. On June 7, the expulsion of the young began, and by June 27 all the females had become spent.

The viviparity, the relative scarcity of males, the great disparity in the size of the sexes and various other facts regarding this species are well known, although I have been able to find no adequate account of some of the most interesting phases of its life history. The principal object of this note is to call attention to the remarkable prolificacy of this little fish, which probably has few parallels among viviparous vertebrates.

The young are expelled in lots of 1 to 5 at short intervals, and the entire brood is delivered in the course of one and a half to three hours. The young swim readily and actively immediately after expulsion. Their length at birth is 8 to 9 mm. The progeny of one mother fish forms a very sizable school; and it was this that suggested the taking of an accurate family census. On one moribund fish 5 cm. long, that had apparently succumbed from inability to expel her young, a Cesarian operation was performed, and 33 living and 51 dead embryos were taken. Other fish 4.5 to 5 cm. long were killed, and counts of the fully developed young were made, the numbers ranging from 85 to 134, the average for all fish examined being exactly 100.

The production of two broods in a season has been suggested by the fact that young are born in spring and also in late summer. This may indicate only a protracted breeding season; but in the fish now under observation there are conspicuous ova which might easily reach full development in six to eight weeks, and fish from the same locality which I examined 22 years ago contained large embryos on August 11. If there are later broods, as I am now inclined to believe, this might account for the marked difference in the average number of young ascertained to be produced by fish observed in June, 1912, and by fish of same size and from same stream in August, 1890, the average for the former being 100 and for the latter 24 (the extremes being 18 and 30). Inasmuch as a second lot of ova would have to attain a certain degree of development while the abdomen was crowded with embryos, it might easily happen that fewer eggs would come to maturity and be fertilized than in the case of the first brood. This may afford a clue to the statement of the late Professor Ryder that "viviparous forms like the cyprinodonts have comparatively few ova, and the number may be as few as 15 or 20 in such a form as *Gambusia.*"[1]

An interesting observation is the cannibalistic tendency of the parent fish. Notwithstanding other food was present, the adults showed a pronounced fondness for their offspring, and began to feed on them soon after they were born. In order to save the young, it was necessary to retain the adults in a wire cage through the meshes of which the young could escape into the aquarium. One fish 4.8 cm. which was transferred to a special receptacle produced 85 living, healthy young, and devoured about half of them during the second night. Another fish that was under observation chased assiduously her first born as soon as it was expelled.

H. M. Smith

Washington, D. C.,
July 1, 1912

[1] Bull. U. S. Fish Comm., 1883, p. 196.