EMPUSA MUSCAE versus *MUSCA DOMESTICA* L.

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(With Plate XXI.)

THE biological control of insect pests has received considerable attention for some years past, and success has without doubt accompanied research work along these lines. The common house fly (*Musca domestica*, L.) is more than ever now, because of additional proof furnished by more recent investigations into their responsibility as carriers of wound infections, recognised as an important source of danger to public health.

The most destructive disease, to which the house fly is subject, is that caused by the fungus *Empusa Muscae*. The question whether this fungus may be used in the control of this pest is of decided interest, and has received more or less careful attention from writers, as well as from investigators.

It has already been pointed out in an earlier paper¹ that it is an easy matter experimentally to spread the disease among flies in captivity. That is to say material from flies recently dead from the disease produced infection in others brought into contact with the spores of *Empusa*. Yet, fatal as the disease is among flies, it was for long a matter of conjecture whether it really constituted an important factor in the death of flies. No doubt the very fact of its being fatal, coupled with the number of dead flies to be seen every year towards autumn, helped to attach an importance to this disease, which a close study failed to confirm. Close observations made in places, where flies are present by thousands throughout the season, indicate that perhaps one in a thousand flies is killed naturally by the disease,—in some instances even a smaller proportion. The determination of the mortality among flies from this disease is comparatively simple, since, as a rule, the fly killed by *Empusa* is and remains attached to

¹ Güssow, H. T., "*Empusa Muscae* and the Extermination of the house fly." *Rep. Loc. Govt. Board, on Publ. Health and Medical Subjects.* New Series, No. 85, p. 10.

the substratum where it died. In the localities referred to, many thousands of flies frequented the places daily, but only from 1 to 4 at the most of newly deceased flies' bodies were observed on any one day during a period of 22 days. Hence in nature this fungus does not appear to account for the general disappearance of flies in winter.

Though I am quite satisfied that one cannot speak of this disease as the cause of an epidemic among flies, and though, endemically, it may be present every season, the fact that it is so fatal to individual flies made it desirable to experiment with the fungus with a view to spreading the disease more widely and rapidly. This might be done were it possible to grow the fungus conveniently in artificial culture, and use it in the manner of, for instance, D'Herelle's *Coccobacillus acridiorum*, pathogenic to grasshoppers.

Methods of securing artificial cultures have been tried by a number of observers, and because of some recent publications on the subject, which, perhaps, have not come to the attention of scientific workers, it will not be out of place to discuss the opinion of these writers here. In the last note which I prepared (*loc. cit.*), I briefly dealt with the investigations of Mr Edgar Hesse, on the subject of culture of *Empusa*.

The seventh report of the same Board (No. 102, 1914) contains further contributions to the subject under discussion. In the letter of transmittal Dr Newsholme, the medical officer of the Board, briefly reviews the original contributions in the report. We now take exception to the statement that the *Empusa* "is known to be the cause of much of the mortality among the house flies during the autumn months," because of careful observations on the rate of mortality. Notwithstanding my friendly criticism of Mr Hesse's 1912 work in the sixth report, his work receives much support from Dr Bernstein who was appointed by the Board "to supervise and control Mr Hesse's investigations." It must be pointed out here that Mr Hesse claimed and "proved" (!) "the possibility of obtaining from spores of *E. Muscae* derived from flies which had died of the disease, a culture which, under suitable conditions as to moisture and food material, gave a luxuriant growth in a few days, followed by the production of spores. These spores when mixed with syrup and fed to normal flies, induced in them a rapidly fatal disease, which, as judged by the naked eye and by microscopical examination, was indistinguishable from the disease found in the flies attacked by *Empusa Muscae* in the ordinary course." Mr Hesse, be it noted, refers to luxuriant growth and production of spores, but does not here state what growth or spores.

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When, through the courtesy of Dr. C. Gordon Hewitt, some original material of Mr Hesse's culture was placed at my disposal, I could, on examination, find nothing else but *Mucor racemosus*, which certainly produced after a few days a luxuriant growth of the same fungus: we also observed "spores," but not *Empusa* spores. Since that time experiments have been made with spores of this universal mould fungus on living flies, but, notwithstanding an ample supply quite sufficient to fatten them, they died of starvation; but under no circumstances was death due to *Mucor racemosus*, since sound flies, kept under identical conditions minus the fungus, died about the same time from the effects of confinement. Mr Hesse solved the difficulty apparent in this instance by concluding that this fungus was polymorphic, and "that *Empusa* was merely a parasitic form of the *Mucor*." Mr Hesse supplements this statement by the following paragraph:—

"The idea that the *Empusa Muscae* might be polymorphic now presented itself, and also that this might be the reason that its cultivation had not apparently been successful hitherto. I realised that one should not expect to produce a purely parasitic growth on a dead medium (*sic!*), but that, as in the case of other fungi when artificially cultivated, a morphological change might be expected; and came to the conclusion that *Mucor racemosus*, although a saprophyte, might, when the spore is ingested by the fly, assume a parasitic existence and produce the growth, in the tissues of the fly, known as *Empusa Muscae*, whose spores, when cultivated on artificial media, revert to the saprophytic stage."

It is regrettable to notice the author's attempt to interpret an error of his by proposing a somewhat fantastic theory. Surely before such explanation is necessary, he should be sure that what he recorded as occurring did actually occur, viz.: the growth of *Mucor* from germinating *Empusa* spores. It is so clear that it hardly needs stating that he has confused an adulteration of his "cultures" with what he thought he had produced. The use of an egg yolk medium "in thin layers in a Petri dish, sterilised by steam at 100° C. for one hour on three days in succession," clearly indicates the degree of importance attaching to the author's further results, whatever they may be.

He proceeds then by inserting bodies of flies which had died of *Empusa* during the previous autumn. Control dishes were also prepared. In five days sporangia had formed on the dishes containing the egg medium, whilst the control dishes remained quite sterile. The author, unfortunately, does not indicate whether he placed on his

control dishes dead flies, not infected with *Empusa*, but at any rate collected at a similar date (the previous autumn), which precaution one would naturally expect in controlling the results of the first experiment. It appears that he placed no flies whatsoever on his control dishes, which naturally remained sterile. Had he done so, we are prepared to predict the identical formation of sporangia observed in his experiments with *Empusa* killed flies.

Next he made transfers from his culture, sporangia forming in the secondary culture. "Meanwhile," he states, "the fungus of his secondary culture, which in every respect was identical with *Mucor racemosus*, was transferred to a syrup made of sterilised sugar and water, and put aside for experiments." How long he does not say. Nor whether the spores had time to germinate in the sugar solution in the "meantime."

Now comes the most surprising effect! In his experiments Mr Hesse uses "flies all bred from insects in confinement"; in June "the chrysalides of the greater and lesser flies commenced hatching out" (? *Musca domestica* L. the common house fly, and *Fannia canicularis*, L. the lesser house fly). In his first experiment all negative results were secured, owing apparently to absence of plum jam. Notwithstanding an earlier statement by the author that many insects became entangled in the sticky mess of plum jam, in the second experiment this was used together with, though not specifically so stated, a filter paper saturated, as in the first experiment, with a syrup in which had been incorporated spores (we take it *Mucor* spores). By the fourteenth day the first deaths were noticed; by the twenty-first day all flies were dead. "All presented typical outward (*sic*) appearances of infection with *Empusa Muscae*." No control was made in this case, he states.

In experiment three,—a repetition of No. 2,—the first deaths occurred in four days, and by the eleventh day all were dead. Cause of death is not stated.

In the fourth experiment there was a control with flies not fed with "spores," and some fed with spores from the fourth generation of *Mucor*. "The results were the same" (as which?). In the control "no deaths occurred and the flies appeared to be normal four weeks later." This is most remarkable; and, if Mr Hesse had not reported this as an actual observation, I should feel inclined to challenge the accuracy of these observations.

Cultures were then made by this indefatigable worker from "flies which had died in the cages with the naked eye appearance of infection

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with *Emmusa Muscae*." These resulted in a prolific growth of *Mucor racemosus*. Why, I wonder, the cautious expressions of "outward" and "naked eye" appearances? One must instinctively ask:—Was *Empusa Muscae* present, or did it only appear to be present? A statement of this most important fact is carefully avoided. Why?

Dr Bernstein thus adds his opinion: "There can be no doubt than that Mr Hesse has succeeded in producing a growth, ingestion of the spores of which results in the death of all the flies from *Empusa Muscae*." One cannot but feel greatly surprised at such a statement from the nature of the research work quoted.

After consultation with Dr Copeman and Mr Ramsbottom (of the Nat. History Museum) the investigators, Dr Bernstein states, agreed on this fact. Here I should wish to ask:—Did they agree on the fact that the flies dead from *Empusa Muscae* were the flies used, or did they merely agree that all the flies, presumably submitted for examination, were dead from *Empusa Muscae*?

Dr Bernstein now steps in the breach and tries a number of experiments. Cage 1 was not interfered with; Cage 2 received a paper saturated with sterilised syrup of cane sugar; Cage 3 received in addition a paper saturated with syrup containing the spores of *Mucor hiemalis* supplied by Mr Ramsbottom. Cage 4 contained a paper saturated with syrup containing spores of *Mucor racemosus*, "which had been cultivated on egg yolk from *Empusa Muscae* (*sic*). The cages were kept in a warm room (in whose charge, or under whose observation, we would gladly have seen stated). Abnormal changes only occurred in Cage 4. In fourteen days 75 % of the flies were dead. Mr Ramsbottom, examining one of the flies from this cage, expresses his opinion that the manifestations were identical with those of *Empusa Muscae*. No fly died in the other cages from *Empusa*.

The general conclusions are stated as follows:—"It would seem then that there could be no doubt that the deaths of the flies in Cage 4 were due to a fungus indistinguishable from *Mucor racemosus*, but which can be readily cultivated in great quantities from the bodies of flies dead of *Empusa Muscae*." This is more correct; we note "from the bodies of flies dead of *Empusa*"; what rôle did the *Empusa* play therein?

Empusa Muscae spores under no circumstances have produced in the cultures made by myself and many other workers anything else but what belonged exclusively to that fungus; certainly never anything like *Mucor racemosus*. *Mucor racemosus*, or *Mucor* resembling what

may now be somewhat loosely called *Mucor racemosus*, was readily secured from the bodies of any dead fly. We cannot, of course, doubt the correctness of the recorded observations; but we are of the opinion that if a *Mucor* proved fatal to flies in the manner described in these experiments, it must have been one of the pathogenic types or a different species altogether. We expect close diagnostic studies of the pathogenic organism would soon establish the identity. But we do absolutely question that *Mucor* develops from an uncontaminated *Empusa* spore. We are glad to note that Mr Ramsbottom has now become interested in clearing up this somewhat involved research work; and we quite agree with him that—if Mr Hesse's assumption is correct that *Empusa* is a polymorph—a fundamental biological principle would be absolutely overthrown.

The critic, they say, but assumes the rôle when he has failed in producing good or marketable merchandise in his own line. That I have assumed the critic's rôle, I may not deny: in what follows, I would play a constructive part, with what success I modestly leave in turn to my critics to say.

I wish, therefore, in concluding to record some of my cultural experiences and other observations on *Empusa Muscae*, which I have not hitherto published. This may assist in simplifying the apparent difficulties which Mr Hesse had in securing an uncontaminated growth from *Empusa* spores.

In my last paper (*loc. cit.*) I described briefly a method of successful auto-infection of flies coming into contact with flies recently dead from *Empusa*. By this means one has under one's own control the securing of fresh material of the fungus for a considerable period. By placing a fly showing the fresh belts of fungus growth on a pillar made of plasticine, this latter can be bent and twisted under the microscope into any position. When carefully adjusted one can observe the discharge of spores very readily. These fly in every direction and for distances of about seven centimetres.

Hence this fact was used for obtaining spore cultures quite easily, and uncontaminated. A series of clear coverglasses was placed at the bottom of a sterile jar, which was closed with an ordinary cork. A fly which was observed to be surely "infected"—experience having shown the incipient symptoms of infection, viz. sluggishness, increase in volume and lightening in colour of abdomen, and a peculiar reddish colour, a dirty brick red, of the "eyes"—was pinned on to the cork from below, so that, when the stopper was replaced, the fly was securely held above

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the coverglasses underneath. Spores were freely shed and collected absolutely pure.

The usual methods used in spore cultures were then employed, but the results were not satisfactory. I then used for a medium various animal fats—a droplet of butter, of dripping, of lard, etc. These were exposed to the spore bombardment, but no more appreciable results were obtained. Moreover, these substances were found to be very unsuitable for microscopic use owing to their annoying refractive indices and their opacity. How much more unsatisfactory must the egg yolk medium—stone hard, no doubt, from the method of treatment given by Mr Hesse—have proved itself. I also tried sterile asparagus tips—mainly because of the known lecithin contents of this plant—but no satisfactory results were achieved on it either.

I then obtained interesting results from the use of a mineral fat, i.e. common vaseline, as used for ringing slides in hanging drops. This medium was sterile to begin with and made beautiful clear hanging drops or surfaces. A small quantity was placed with a scalpel on a clean coverglass and gently heated until evenly spread like a film over the coverglass. When exposed to spore-shedding, the number of spores desired thereon was easily adjusted by the removal of coverglasses after one, two, three or more minutes' exposure. The coverglasses were then inverted on hollow ground slides and ready for examination.

To obviate any misunderstanding, here let me remark parenthetically that, in the description following, I am not detailing the result of any one particular experiment, but am summarising the nett results of some hundreds of observations.

Germination began very shortly in every instance. The plasmatic mass which surrounds the spore when shot from the conidiophore appeared to remain in a liquid or semi-liquid form; it never showed the wrinkled appearance generally observed, and so often figured. The first symptoms of active life appeared about one hour after the spores had been shot on the medium. Never at any time did the original spore produce a germinal tube; at first there appeared something very much like a germinal tube in the shape of a small "exdentation" of the cell wall of the mother spore. This gradually elongated, but assumed, from ten to fifteen minutes later, a flask or clubshaped appearance. About an hour later it was recognised with certainty as a secondary spore, the nucleus of the first spore having taken up its position in the now forming secondary spore. As this spore grew, it, together with the mother spore, presented a dumb-bell shape. Where joined

to the mother spore the "handle of the dumb-bell" elongated somewhat, and the secondary spore also appeared surrounded by a plasmatic substance—just as did the mother spore—but in very much smaller quantity.

Immediately after the secondary spore was formed, it actually did germinate. It should have been said that in the mother cell the "exdentation" occurred on almost any position of the spherical body. But with the secondary spore, germination, as opposed to the "exdentation" of the mother cell, took place at the end towards the mother cell. Indeed what appeared at first as a rather long "handle to the dumb-bell" was later recognised as a mycelial thread, or, more correctly, tube growing into the cavity of the old spore. Naturally in many cases the secondary spore often separated from the mother spore.

The germ tube was seen to branch, and the contents of the spore to be slowly used up, while the mycelium grew in size. This produced sparse, short, stout branches at no special points. Later on a second germ tube grew from the secondary spore body; on rare occasions there were three in all. The growth made slow progress thereafter, becoming detached from its spore shell, and assuming shapes and sizes similar to the mycelial portion observed in the fat body of the fly. We have not been able to see a true tertiary spore in *Empusa Muscae* after germination took place, although peculiar club or flask shapes occasionally appeared which resembled spores; but they could not, on examination, be determined as such.

The growth remained pure all the time and made progress for 28 days on this medium. Then signs of disintegration appeared, and the mycelium became vacuolated more and more, less sharp in outline, and later collapsed. This is, so far as I know, the longest time a growth has been maintained outside a fly; but we cannot regard it as a successful culture yet. Every effort to continue growth failed, no doubt because it normally takes place in nature in the living fly body.

These observations clearly show that Mr Hesse's *Mucor racemosus* was nothing else but an impurity, and polymorphism does not occur in this fungus, as represented at one time by *Mucor* and another by *Empusa*.

Every other experiment to continue the development of these spores has failed so far. The absence of nutrients accounts for this; for the various ingredients tried to furnish material for continued growth did not suit the fungus. The main points, from a scientific

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point of view, viz. the hibernation, the question of resting spores, so common in other species of this genus, and of artificial culture,—still remain unsolved. But, while it is realised as possible that the solution of these problems may have other than a scientific value, it appears to me from my observation that we must look, for the control of the fly problem, to other biological organisms—or remove systematically, by all necessary precautions, the insanitary conditions favourable to the breeding of this annoying and dangerous pest.

EXPLANATION OF PLATE XXI.

- Fig. 1. *Empusa* spore shot on vaseline at 9.15 a.m.
Fig. 2. Same spore at 11.15 a.m.
Fig. 3. " " 12.10 p.m.
Fig. 4. " " 12.25 p.m.
Figs. 5, 6. Same spore, a little later.
Fig. 7. Same spore at 4.40 p.m.
Fig. 8. " " 5.20 p.m.
Fig. 9. " " 5.30 p.m.
Fig. 10. " " 6.5 p.m.
Fig. 11. Spore shot on glass slide, mass of protoplasm drying up.
Fig. 12. Same spore as before after 24 hours' germination.
Figs. 13–17. "Involution forms."
Figs. 18–20. Spore germinating into mother spore, and becoming separated from same.
Fig. 21. Proboscis of *Musca domestica* and spore of *Empusa Muscae*, relative sizes.