ON THE SURVIVAL OF SPECIFIC MICROORGANISMS IN PUPAE AND IMAGINES OF *MUSCA DOMESTICA* RAISED FROM EXPERIMENTALLY INFECTED LARVAE. EXPERIMENTS WITH *B. TYPHOSUS.*

By J. C. G. LEDINGHAM, D.Sc., M.B., M.A.
Lister Institute, London.

It has recently been shown by Bacot (1911), some of whose experiments I had an opportunity of confirming (see Appendix to Bacot's paper), that bacilli (*B. pyocyaneus*) taken up by larvae of *Musca domestica* may survive the pupal stage and reappear in the imago.

In view of the importance of this fact, it appeared desirable in the interests of practical hygiene, to ascertain whether an organism such as *B. typhosus* was also able to adapt itself to the conditions prevailing in the larval and pupal interior where presumably it would have to contend with other organisms of a more hardy character.

For the purpose of the experiments detailed in this communication, ova of *Musca domestica* were kindly supplied to me by Mr Bacot.

First Series of Experiments.

Ova of *Musca domestica* were received on 5 Dec. 1910. The larvae were transferred to sterile vessels containing a layer of sterilised sand and supplied with sterilised food consisting of mashed meat, potatoes and turnips. The mash was drenched repeatedly with broth cultures of *B. typhosus.* While the larvae were still feeding, it was noted that the mash had a strongly ammoniacal smell and the question arose whether the *B. typhosus* which had been supplied so liberally to it, was the predominant organism in the mash. Examination of MacConkey plates from an emulsion of the meat showed numerous colourless colonies none of which proved to be *B. typhosus.* They all gave the

characters of an organism frequently encountered throughout this work and which I shall provisionally call *Bacillus "A,"* the chief points of difference between it and *B. typhosus,* being the formation of indole and intense production of alkali in litmus milk following an initial acidity. Pure cultures of this organism gave the characteristic ammoniacal smell given by the mash in which the larvae were feeding. *B. prodigiosus* was also present in the mash.

Examination of larvae before pupation.

Four fully grown larvae were placed in absolute alcohol for 10–15 minutes and then washed repeatedly in sterile broth. Plates prepared from the mashed-up larvae gave profuse growth of colonies of *Bacillus "A,"* and a few colonies of *B. prodigiosus. B. typhosus* was not recovered.

Examination of pupae.

On 12 Dec. twelve pupae were found. These were transferred to sterile sand in a sterile flask. The remaining larvae were washed several times in 2 % lysol followed by sterile broth and finally transferred to another sterile bottle to pupate. For the bacteriological examination of pupae various methods of disinfecting the exterior were employed, similar to those mentioned in the Appendix to Mr Bacot's paper (p. 73) but it was found impossible to obtain sterile washings before the final mashing-up process. In any case, the plates prepared from the triturated pupae gave profuse growth of colonies of *Bacillus "A." B. typhosus* was not recovered.

The direct method of examining the bacterial content of the pupal interior was then devised (see Appendix to Mr Bacot's paper) and gave the following results in a series of pupae.

Pupae (one-day old) examined by direct method.

No. I. Sterile.
No. II. Profuse growth consisting of *Bacillus "A."*
No. III. Profuse growth consisting of *Bacillus "A"* and *B. prodigiosus.*
No. IV. Sterile.
No. V. Sterile.
No. VI. Only one colony on plate, viz. *Bacillus "A."*
No. VII. Sterile.

Pupae (two days old) examined by direct method.

No. I. Sterile.
No. II. Few colonies, all *Bacillus "A."*
No. III. Profuse growth of colonies of *Bacillus "A."*
No. IV. do.
No. V. do. + *B. prodigiosus* + *B. pyocyaneus.*
No. VI. Mainly *B. pyocyaneus.*
No. VII. Profuse growth of *Bacillus "A."*
In no case was *B. typhosus* recovered from the plates.

Examination of emerged Flies.

The procedure adopted in examining the adult fly was as follows: the fly after being chloroformed was immersed in absolute alcohol for 5–10 minutes and afterwards transferred to a sterile slide where the wings and legs were removed. It was then passed through the flame. The abdomen was opened with sterile needles and the intestine carefully removed and mashed up in salt solution or broth.

Results.

Fly No. I. Emerged on 19th Dec. and examined on the same day: Plates showed profuse growth of *Bacillus "A."*
Films prepared from the intestine showed enormous numbers of bacilli.
Fly No. II. (About 10 hrs. old. Had fed on sterile cane sugar and had probably evacuated its intestinal contents.) Plates proved sterile. Microscopically only a very few bacilli were observed in stained films.
Fly No. III. (24 hrs. old.) Plates sterile.
Fly No. IV. (24 hrs. old.) Plates showed *Bacillus "A," B. prodigiosus* and *B. pyocyaneus.*
Fly No. V. (48 hrs. old.) Pure plates of *Bacillus "A."*
Fly No. VI. (do.) (do.)
Fly No. VII. (do.) *Bacillus "A"* and *B. pyocyaneus.*
Fly No. VIII. (do.) One colony of *Bacillus "A."*

In all cases where the plates showed profuse growth of colourless colonies, a large number were picked off and carefully examined on the chance that the typhoid bacillus might be recovered, but in no case was a successful result obtained. All the colonies picked off proved invariably to consist of *Bacillus "A."*

Second Series.

Ova were received on 4th and 5th Jan. 1911 from Mr Bacot, and within 12–24 hrs. all had hatched out. On this occasion cultures were made from the egg-shells and some of the newly emerged larvae, as the negative results obtained in the first series of feeding experiments with *B. typhosus* had given rise to the suspicion that this micro-organism had only a very small chance of survival in competition with organisms contaminating the exterior of the ova and which were probably ingested at a very early period by the newly emerged larvae.

The plates showed profuse growth consisting of the following organisms, *B. proteus vulgaris, Bacillus "A," B. prodigiosus* and a *Streptococcus*. The larvae were fed with a sterile mash and kept at 25° C. until the 9th Jan. when several full-grown individuals were found.

These were washed in carbolic solution and finally transferred to a fresh sterile vessel containing sterile sand to which a liberal supply of typhoid broth cultures had been added. One, however, was not placed in the typhoid chamber but was retained for bacteriological examination. Plates prepared from it gave a growth consisting of *Bacillus "A," B. prodigiosus* and a lactose fermenter (*Bacillus "B," No.* 3, vide Table I). On 10th Jan., one larva was removed from the typhoid chamber and examined after careful disinfection in carbolic solution. The plates consisted mainly of *Bacillus "A"* and *B. proteus vulgaris* but one colony was found to consist of *B. typhosus*. Whether this organism actually came from the larval interior could not be decided, as the broth in which the larva had been washed after disinfection gave after incubation a growth which consisted of *Bacillus "A,"* and *B. proteus vulgaris.*

Examination of Pupae.

Pupa No. I. (12 hrs. after pupation.) Plates gave pure growth of *Bacillus "A."*

Pupa No. II. (12–24 hrs. after pupation.) Profuse growth of *Bacillus "A."*

Pupa No. III. (3–4 days after pupation.) Plates consisted of *Bacillus "A," Bacillus "C"* (see Table), and *Bacillus "E"* (see Table).

Pupa No. IV. (4 days after pupation.) Plates consisted of *Bacillus "A"* and *B. prodigiosus.*

In no case was *B. typhosus* recovered from the pupae.

One adult fly was examined after it had fed on sterile sugar for two days.

The plates proved sterile.

Third Series.

On 24 Jan. 1911 a new batch of eggs was received and an attempt was made to sterilise the eggs before the emergence of the larvae, but before sterilisation a sample of the eggs was taken for bacteriological examination. The organisms isolated were chiefly those marked in the Table as *Bacillus "B" No.* 3, *Bacillus "B" No.* 2, and *Bacillus "D."* The remaining eggs were placed in 5% lysol for 2½ mins., washed repeatedly in saline solution and allowed to dry as far as possible. Sterile human blood was then given them but unfortunately the eggs became entangled in the fibrin meshes and no larvae emerged.

Final Series.

On 25 Jan. another batch of newly emerged larvae was received from Mr Bacot. *These had developed from eggs which had been disinfected by a short sojourn in lysol.* The young larvae had been placed on a sterile agar slope which remained sterile. Human blood mixed with typhoid bacilli was spread on the agar and this process was repeated, the larvae being transferred to a fresh agar slope with blood every day.

Examination of larva.

On 29 Jan. one larva was removed from the slope and placed in 5% lysol for 5–10 mins. After successive baths of saline solution and absolute alcohol the larva was mashed and plated.

The plates yielded what appeared to be a pure growth of typhoid-like colonies.

Seven of these were picked off, all of which proved to be *B. typhosus.*

Films made from the mashed-up larva also showed numerous typhoid-like organisms with a few large diplococci.

Further examination of larvae.

On 31 Jan. four larvae were found dead in the condensation fluid of the agar, the excess not having been removed by an unfortunate omission. Only one larva survived. Two of the dead larvae were placed in 2% lysol for 10 hours and then plated.

B. typhosus was recovered in pure culture from each.

The surviving larva was placed on fresh sterile sand and pupated between 3rd and 4th Feb.

Examination of pupa.

On 6 Feb. the pupa was examined by the direct method. The colonies on the plates were few but all of them proved to be *B. typhosus.* They were agglutinated by an anti-serum in dilution of 1 in 2000.

Summary and Conclusions.

Although typhoid bacilli were liberally supplied to larvae of *Musca domestica,* all attempts to demonstrate *B. typhosus* in the pupae or imagines were unsuccessful, until recourse was had to disinfection of the ova. After this preliminary disinfection both larvae and pupae gave pure growths of *B. typhosus* but hitherto it has not been possible to examine the imagines.

In the experiments with unsterilised ova great difficulty was experienced in determining whether *B. typhosus* was present in MacConkey plates owing to the almost invariable occurrence of the colourless typhoid-like colonies of the *Bacillus "A"* which was evidently an organism thoroughly adapted to the conditions prevailing in the interior of the larvae, pupae and imagines.

The fact also that this organism like *B. typhosus* fermented mannite without the production of gas, rendered the search for *B. typhosus* still more difficult.

By the employment of sorbite—in place of lactose-media, a preliminary differentiation might have been possible but was not considered practicable in the first place owing to the expense of sorbite, and secondly owing to the fact that the fermentation of sorbite by *B. typhosus* though constant, is sometimes delayed. The most satisfactory plan was to pick off as many colonies as possible and inoculate them on litmus-milk. *Bacillus "A"* invariably rendered litmus-milk intensely alkaline.

From the practical point of view the main conclusion to be drawn from the experiments detailed in this communication is that the typhoid bacillus can lead only a very precarious existence in the interior of larvae or pupae which possess, at least in so far as these investigations warrant, a well-defined bacterial flora of their own.

Even under the highly artificial conditions of the final series of experiments, it was not possible to decide whether the *B. typhosus* though recoverable from the pupa was really actively multiplying in the pupal interior or gradually dying out. There was some indication that the latter was the case, as the typhoid colonies recovered from the pupa

in the one successful instance, were extremely few in number, while the larvae which had been feeding on *B. typhosus* contained enormous numbers as evidenced both by cultural and microscopical examination.

Since the above experiments were concluded, some further researches by Graham-Smith (1911) on the carriage of bacteria by flies have appeared in the form of a Local Government Board Report. This author has succeeded in recovering *B. anthracis* from blow-flies bred from larvae fed on meat infected with spores of this organism, but has failed to recover *B. typhosus* or *B. enteritidis* under similar experimental conditions.

TABLE I. *Characters of the chief organisms isolated from ova, larvae, pupae and imagines.*

(*B. typhosus* inserted for comparison with *Bacillus "A."*)

	Motility	Liquefaction of gelatine	Lactose	Glucose	Mannite	Dulcite	Saccharose	Sorbite	Litmus milk	Indole	Adon.	Inul.
B. typhosus	+	–	–	A	A	–	–	A	A	–		
Bacillus "A"	+	–	–	A	A	–	–	–	A – alk.	+		
B. prot. vulg.	+	+	–	AG	–	–	AG	–	AC pept.	+		
B. prodigiosus (α)	+	+	–	AG	A	–	A	A	AC pept.	–		
do. (β)	+	+	–	AG	A	–	AG	A	AC pept.	+		
Bacillus "B" No. 1	–	–	AG	AG	AG	AG	AG	AG	AC	–		
do. No. 2	+	–	AG	AG	AG	–	AG	AG	AC	–		
do. No. 3	–	–	AG	AG	AG	AG	AG	AG	AC	+	AG	–
Bacillus "C"	+	+	–	AG	AG	–	AG	–	Pept.	+		
Bacillus "D"	+	–	–	AG	AG	–	AG	AG	AC	–		
Bacillus "E"	–	–	–	A	–	–	–	–	A	–		

Notes on the organisms in Table I.

Bacillus "A." Compare with organism marked 4 B isolated by Morgan from faeces of children (see Morgan and Ledingham, *Proc. Roy. Soc. of Med.* Epidem. Sect. March, 1909, Table I). Also isolated by the writer from faeces of typhoid convalescents and examined by Morgan (see *Journ. of Hyg.* Vol. XI., No. 1, April, 1911, Table II. Organisms B 20—B 25). They are not agglutinated by typhoid or dysentery serum.

"B" No. 1—*"B"* No. 3. Lactose-fermenters, belonging to Groups III and IV in MacConkey's scheme (see MacConkey, "A Contribution to the Bacteriology of Milk." *Journ. of Hyg.* Vol. VI., 1906).

Bacillus "D." Compare with organisms marked No. 12 and No. 13, isolated from faeces of children by Morgan (see Morgan and Ledingham, *ibidem*).

The occurrence of *B. pyocyaneus* is explained by the fact that Mr Bacot had been experimenting with this organism. The ova had become contaminated by fly excreta containing this organism among others.

REFERENCES.

BACOT, A. W. (1911). The persistence of *Bacillus pyocyaneus* in pupae and imagines of *Musca domestica* raised from larvae experimentally infected with the bacillus. *Parasitology*, IV. 68.

GRAHAM-SMITH, G. S. (1911). Further observations on the ways in which artificially infected flies carry and distribute pathogenic and other bacteria. *Reports to Local Govern. Board*, 1911. New Series, No. 53, p. 31.

https://doi.org/10.1017/S0022172400016788 Published online by Cambridge University Press